PART I
FINAL REPORT

A STUDY OF POST-RECUMBENCY ORTHOSTATISM AND PROPHYLACTIC MEASURES FOR PREVENTION OF THIS PHENOMENON

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FINAL REPORT

A STUDY OF POST-RECUMBENCY ORTHOSTATISM AND PROPHYLACTIC MEASURES FOR PREVENTION OF THIS PHENOMENON

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I. INTRODUCTION

Throughout the eons of time man has shown remarkable adaptability to virtually every type of environmental change. However, each of these changes has been faced with at least one factor remaining constant. That factor is the presence of gravity. As a result of the ever constant presence of a 1 G (gravity) environment, man has evolved the necessary anatomic structures and physiologic mechanisms to permit him to live and function effectively. If it were not for a perfectly structured musculoskeletal system, he would be little more than an intelligent jelly-fish. However, the development of a bony structure which allows him to lie down, sit or stand had to be accompanied by an evolution of the neurovascular system which would allow smooth maintenance of blood flow to vital organs and return of blood to the heart and lungs at an adequate rate in spite of body position. The need for this gravity adaptive system within an unchanging 1 G environment can best be understood by considering the hydrostatic effect of gravity on the vascular system in the horizontal (supine) and the vertical (erect) postures.

The vascular system can be simulated with a distensible mercury filled bag with a length approximately seven times its width (See Figure 1). The internal pressure of the system is maintained by a compressor. If the pressure exerted by the compressor is maintained constant at all times, then the pressure exerted on the internal wall at any point will be equal to the sum of filling

pressure, compressor pressure, and the pressure exerted by the weight of mercury at that point. The sum of these pressures will be modified by the compliance of the bag. The weight or force exerted by the mercury will be equivalent to the height of the mercury column over that point. This force is hydrostatic pressure. In the supine or horizontal position, pressure will be equivalent to the width of the column or 12 mm. Hg. In the erect or vertical position it will be equivalent to the height of the column or 85 mm. Hg. Thus, the effect of gravity on this simulated vascular system in the vertical position would be approximately seven times as great as in the horizontal position. Therefore, even if the gravitational environment is constant, its effects on the simulated or real vascular system are not. They are dependent on whether the force is exerted in a cephalocaudal or anterior-posterior direction. In the supine position the effect is so minimal as to cause little distortion of the bag or vascular system. On the other hand, in the erect position, considerable pressure is exerted on the internal walls of the lowest portion of the bag or vascular system. Since the bag is quite distensible, this results in great distortion. The greatest amount occurring in the most dependent areas. If the volume of mercury is constant, the distention of the dependent portion of the bag and resultant shift in the mercury will result in underfilling and perhaps collapse of the more cephalad segment of the bag.

The pressure at any point within the circulatory system is the resultant of three factors: the static filling pressure (83,178);

the dynamic component which is due to flow and resistance; and the hydrostatic pressure caused by the force of gravity. The pressure within the arterial tree is predominantly determined by variations in blood flow and resistance to flow. Although pressure difference due to the hydrostatic column is measurable, the volume of the arterial tree is minimally altered by this force. This is true because of the muscular arterial walls. On the other hand, hydrostatic pressure has striking effects on both venous pressure and volume. In the upright posture, the venous pressure at foot level is approximately 85 mm. Hg. higher than at heart level. This is seven times the hydrostatic effect which is present in the supine position. Because of the marked distensibility of the venous bed, this large pressure increase results in pooling of approximately 500 ml. of blood in the lower extremities (8,80,89,91,194,214,225). The degree of pooling is affected by the environmental temperature as well as by other constricting influences (62). If the orthostatic posture is maintained for long periods without leg activity, there will be a slow continuation of dependent pooling for many hours (80,214,219,225). This is partly due to stress relaxation of veins (3,62), but is also the result of extravasation of plasma filtrate. This occurs as a result of the elevated capillary filtration pressure and may be detected by changes in hematocrit and plasma protein concentration (12,31,213,225). The resultant decrease in plasma volume may amount to 11-15% of the total blood volume.

Sjostrand found that most of the blood displaced into the dependent areas was derived from the intrathoracic compartment (173). There was a 25% decrease in central blood volume. This is in agreement with the finding of other investigators (46,84, 85,113,119,150,155,195,217). Thus, the intrathoracic vessels serve as an important reservoir which can fulfill the left ventricular requirements for filling pressure during the early stages of orthostasis when cardiac output exceeds venous return. The decrease in central blood volume is also reflected by diminished heart size (123,154). The decrease in cardiac volume has been estimated to be 130 cc. in normal man and the change is most pronounced in the atria and large veins (151).

The resultant reduction in end-diastolic volume leads to a decrease in stroke volume to approximately 50-60% of recumbent values (20, 21,154,210). Heart rate increases but not enough to maintain cardiac output, which drops to 60-80% of the recumbent value (140, 180,195,211). The decrease in blood flow is reflected in an increase in peripheral oxygen extraction (20,21). As a consequence of these changes, arterial pulse pressure narrows, primarily due to a rise in diastolic pressure (77,168,208). The primary importance of central blood volume in these changes is confirmed by the prevention of a fall in cardiac output by various methods including the G-suit and water immersion (9,10,11,56,152,173,216). Thus, orthostasis can be considered as a functional hemorrhage into the dependent vascular bed (79,80).

This shift of blood out of the central compartment results in a fall in right atrial pressure (21,30,113). Pressures above the right atrium fall below zero (48,157,158,223). As already noted, pressure rises in the lower extremities. Thus, there must be a point within the vascular tree where alterations in body position cause no effect. This point is known as the hydrostatic indifference point or H.I.P. (207). It has been found to lie in a few centimeters below the diaphragm in upright human subjects (58). This suggests that distending pressures in the liver are minimal. Thus, orthostatic pooling is unlikely to occur in the vascular bed of the liver. Liver blood flow has been shown to decrease (78,156). In addition, the tone of the abdominal muscles acting upon the soft tissues of the gastro-intestinal tract creates a water jacket effect which cancels out the intravascular hydrostatic effect within the abdomen (47,163). Thus, the loss of central blood volume is primarily due to a shift of blood into the venous compartment and tissues of the lower extremities, not into the viscera.

When this volume shift occurs, the concomitant drop in stroke volume and cardiac output would be much more extreme if it were not for certain defensive mechanisms which are called into action in an attempt to maintain homeostasis. The first line of defense is the baroreceptor system. The arterial baroreceptors lie in the carotid sinus and aortic arch (13,49,92). The activity of these receptors is decisively influenced by the pulse pressure (92). In terms of postural effect, the carotid sinus baroreceptors

are probably more important than those in the aortic arch. This is due to their proximity to the brain as well as to their greater distance from the hydrostatic indifference point. There are also widespread receptor areas in the intrathoracic portion of the low pressure circulation (13,92). The low pressure system is more distensible and small pressure changes due to orthostatic blood volume shifts are accompanied by large changes in wall distention (60). These receptors may be more important to orthostatic homeostasis than the arterial baroreceptors. This is suggested by the fact that the increase in heart rate produced by going from the supine to the sitting position is less pronounced than that which occurs on changing from the sitting to the standing posture (98,128). The change from recumbency to sitting is associated with a greater decrease in hydrostatic pressure at the level of the carotid sinus than is the case on changing from the sitting to the standing position. However, the change from a sitting to a standing posture is associated with a greater drop in intrathoracic blood volume. Additional support for the importance of the low pressure baroreceptors is provided by the fact that measures which maintain central blood volume decrease the reflex effects of orthostasis. Examples include leg wrapping, antigravity pressure suits, water immersion and transfusions (10,11,56,94,173).

The diminished stretch of the baroreceptors results in increased sympathetic nervous tone which increases heart rate, thereby compensating partially for the diminished stroke volume. In addition,

peripheral vasoconstriction occurs. This increased activity of the sympathetic nervous system can be demonstrated by the increase in catecholamine excretion (51,95,185,197). This is not seen in patients with autonomic insufficiency (127). Peripheral vasoconstriction assists in blood pressure maintenance by increasing arteriolar resistance and by diminishing vascular volume to a small extent. Although it is well established that peripheral veins are capable of very forceful contractions (2,27,54,62), their tone is increased minimally and only transiently in response to the upright posture (32,165,166,190). It has also been shown that the capability of veins to displace volume becomes progressively smaller at higher venous pressures (1,36,62). This suggests that the stimulus required to produce vasoconstriction must be much stronger than that which is required to produce resistance vessel constriction.

Thus, venoconstriction does not provide the means for restoration of the central blood volume during orthostasis. Since capacity cannot be adjusted to volume, volume must be adjusted to capacity. This important function of volume adjustment is performed by the baroreceptors. Decreased baroreceptor stimulation, as induced by diminished vascular filling, results in sodium and water retention and increased stimulation results in sodium and water diuresis (50,52,59,60,110,188). Orthostasis is one of the many factors which alter intravascular filling and thereby modify A.D.H. release. In addition, aldosterone secretion is increased

by the upright posture. This response is absent when the subject stands in a water bath, thereby preventing the fall in intrathoracic volume (69). Although the orthostatic posture is associated with a fall in renal plasma flow and glomerular filtration rate, the correlation of these changes with alterations in sodium and water handling has not been consistent (60,174,220). Recent studies by Stahl suggest that an alteration in renal arteriolar perfusion pressure at the distal afferent arteriole may play a direct role in sodium and water handling by the kidney (176). The presence of an unidentified third factor has also been suggested (29). Since man spends many hours in the upright posture, it would appear that vascular volume is adjusted to mean vascular capacity. Thus, the individual with a large capacity in the upright posture will have an above average blood volume. On assuming the supine posture his stroke volume, cardiac output, and venous pressure will be higher than in his more average peer. This is manifested quite well in patients with massive varicosities (37,135), as well as in some patients with idiopathic orthostatic hypotension (22). When the mean vascular capacity is chronically reduced, the converse is true. This can be seen with prolonged bedrest in healthy subjects (41,42,143,144,145) or with prolonged water immersion (70,74, 105). In these situations blood volume is said to be reduced. Thus, it would appear that recurrent orthostatic stress is necessary in the maintenance of a blood volume adequate for all postural changes. The great sensitivity of the volume regulating mechanism

presents a striking contrast to the sluggishness of the venomotor response to orthostasis. This leads to the obvious conclusion that volume is preferentially adjusted to capacity in the normal human.

Although the baroreceptors are critical to orthostatic hemostasis, additional protective mechanisms are present. It has been shown that the vascular smooth muscle of resistance vessels reacts directly to increased vessel wall stretch by active contraction and to diminished stretch by relaxation. This is the so-called Bayliss Effect (17,53). A similar mechanism has not been identified in capacity vessels (78).

Since the hydrostatic indifference point lies below the heart in the upright position, it might be expected that the heart and great veins would collapse as a result of the negative intravascular pressure. This might well occur if it were not for the greater negativity of intrathoracic pressure, particularly during inspiration. An additional factor which tends to move blood toward the heart is the increase in intra-abdominal pressure during inspiration (111,112,141).

The tissues of the lower extremity offer little or no protection from the fluid shifts that occur during orthostasis. Subcutaneous pressures rise only a few centimeters on assuming the upright posture (35,134,219). The rise in intramuscular pressure is somewhat greater, especially in muscles with tight fascia (87,134,219). This rise in pressure is due to the elevated

hydrostatic pressure as well as the plasma water accumulation (219). In addition, there is a higher resting tone of skeletal muscle in the upright posture (87,88,134). The physiologic significance of static muscle tone is poorly understood. However, the active contractions of the so-called 'muscle pump' represent a most efficient mechanism for decreasing dependent blood pooling. When the calf muscles contract, deep veins are compressed, moving their blood content toward the heart (39). During muscle relaxation, venous valves prevent reflux of blood. Also, during relaxation, blood is pulled into the interfascial deep veins from the superficial veins (112,214). This results in a large drop in venous pressure at the ankle level (90,96,97,104,160,209). The degree and persistance of this pressure drop is dependent on the rate of inflow from the arterial tree. This, in turn, is dependent on the degree of vasoconstriction present in the resistance vessels (90). The importance of the 'muscle pump' mechanism can be seen when walking is compared with quiet standing. During walking the increase in foot volume due to the upright posture is less and hemoconcentration due to extravasation of plasma water is less pronounced (126,225).

In spite of the many protective mechanisms, absolute compensation for the upright posture probably does not occur even in the normal man. With continued orthostasis, a progressive decrease in central blood volume is very likely because of slow continuous distention of dependent capacity vessels (stress

relaxation) as well as a continuous extravasation of plasma fluid. If these changes cannot be prevented by the muscle pump, stroke volume and cardiac output will finally drop below a compensable level. These changes are associated with progressive narrowing of pulse pressure and with a decrease in mean arterial pressure. Heart rate continues to rise and vasoconstriction becomes progressively more profound (28,142,157). At some point the latter factor may actually become detrimental to the maintenance of blood flow (122). Vasovagal syncope may occur with an abrupt fall in arterial pressure. This will be associated with bradycardia and ultimate unconsciousness (120,171). If orthostasis is not terminated, the individual may succumb of organ hypoxia or cardiac arrest.

However, the occurrence of vasovagal syncope is associated with dilatation of the skeletal muscle resistance vessels (6,15, 16,30). This may account for the fact that stroke volume and cardiac output do not show a further decrease during the syncopal episode. These parameters remain near levels that obtained immediately prior to syncope (65,212,218). This maintenance of blood flow and venous return affords a degree of sustinance to the individual with vasovagal syncope.

The vasovagal response may be seen in nearly all healthy subjects if orthostasis is maintained for a sufficient duration. The likelihood of its occurrence is affected by many factors. Conditions which predispose are those which either decrease

vascular filling or increase vascular capacity. These include hemorrhage (16,81), heat (162), prolonged recumbency (55,56,145, 198), massive varicosities (23,37), vasodilator drugs (67,121, 218,222), severe muscular excretion (4,130), hypoxia (6,86,131), psychic stress (16,93,172), and water immersion (71,72,138) and sympathectomy (224). Increased tolerance to orthostatic stress is produced by an expanded total or intrathoracic blood volume (94,218). Congestive heart failure is a classic example of this situation (171). Physical training also improves tolerance (4,68).

The trigger mechanism resulting in the pronounced depressor effect in vasovagal syncope is not well understood. It has been suggested that it may be due to continued forceful contraction of an empty ventricle. This is the von Bezhold-Jarisch reflex (13,61,64,92,103,171). It has been suggested that the cholinergic sympathetic dilator system might be responsible for vasodepressor syncope (15,16). However, blockade of this system with atropine simple prevents the associated bradycardia but does not alter the fall in arterial pressure and peripheral vascular resistance (120,215,218).

As noted previously, the incidence of vasodepressor syncope is increased by extended periods of bedrest. The importance of this and other problems associated with bedrest was stressed by a number of clinicians in the World War II era (45,107,109,118, 161,187). The scope of these problems has been summarized in a recent publication (33). The reason for this increased incidence

has been related to diminished intravascular volume (20). It has also been suggested that deprivation from normal gravitational stimuli results in disuse 'atrophy' of the cardiovascular postural reflexes. Medical interest in the problem waned when it became apparent that avoidance of prolonged bedrest or immobilization would prevent the development of postural hypotension as well as other problems associated with bedrest. Thus, in spite of the wealth of knowledge regarding man's response to gravity which is reviewed above as well as in greater detail elsewhere (63), the mechanisms involved in de-adaptation to gravity are not well defined.

With the advent of space flight man is now being subjected to periods of weightlessness comparable in time to periods of bedrest known to produce orthostatic intolerance. In many respects the circumstances of space flight are even more prone to engender orthostatic hypotension on a re-exposure to gravity. Firstly, the astronaut is at zero gravity as opposed to the 1/7 G associated with horizontal bedrest. He is also partially immobilized because of the design restrictions of his spacecraft. His environment and activities are conducive to fatigue and inadequate rest. He undergoes periods of great emotional stress which are conducive to exhaustion and dehydration even if ample time is allowed for rest and ample fluid is provided. This is true even in the presence of an optimally functioning cooling system and in the absence of febrile illnesses during space flight. Dehydration has been shown

to aggravate the tendency to orthostatic hypotension (43). Injuries, which predispose to orthostatic intolerance, are not inconceivable. Finally, at some point during spaceflight or during or after re-entry, the astronaut may be exposed to positive gravitational forces in the head to foot axis, thereby setting the stage for orthostatic hypotension, and its undesirable consequences.

The prevention of "cardiovascular deconditioning" during spaceflight requires a detailed understanding of the mechanisms involved in this adaptation to the absence of gravity. In an absolute sense, this problem cannot be studied until such time as the Manned Orbiting Laboratory is available. However, for reasons stated earlier, absolute horizontal bedrest provides a useful, although admittedly imperfect, analog. Only the degree of effect should differ from the changes that occur in the zero gravity environment. The qualitative response should be quite similar.

The present study was undertaken in an attempt to define the physiologic alterations that result in cardiovascular deconditioning. Bedrest was utilized as an analog to weightlessness.

II. EXPERIMENTAL DESIGN:

A. General

Healthy male volunteers, ages 21-35, served as subjects for the present study. All volunteers were obtained from the Federal Correctional Institution, Lompoc, California by permission of the U.S. Bureau of Prisons. Volunteers were screened by the medical staff of the Federal Correctional Institution to exclude major or chronic health defects. Each volunteer was then subjected to a 70° passive tilt for twenty minutes to exclude the presence of autonomic insufficiency. A tilt table with English saddle was provided to the F.C.I. staff for this purpose.

On completion of the screening procedure, the volunteer was transported to the U.S. Public Health Service Hospital, San Francisco, California. On arrival the volunteers were admitted to the Metabolic Unit where a complete medical history was taken and a physical examination was performed. A twelve-lead electrocardiogram was done as well as a chest x-ray. In most cases a flat film of the abdomen was taken to exclude radiopaque renal stones. Routine laboratory evaluation included C.B.C., urinalysis, serology, fasting blood sugar and blood urea nitrogen. Prior to entry into the study, the 70° passive tilt was repeated without vascular instrumentation.

The plan of study required that each subject be placed on a formula diet forty-eight hours prior to entering the study period. The dietetic aspects of the study are covered under

IV. Metabolic Dietetic Program. During this equilibrium period, the volunteers were instructed in urine and stool collection and intake recording by the metabolic nurse.

The study consisted of two phases, a seven day Ambulatory Phase and a fourteen day Bedrest Phase. Normal ambulatory activity was allowed during the initial week and napping was discouraged. While at bedrest the horizontal position was required at all times with unrestricted movement in this axis. Arm movement was limited to forearm raising with elbows on the bed. One pillow was permitted for head support. Excessive boredom was avoided by the use of reading material, games, radio and television.

Twenty-three volunteers completed fourteen days of bedrest. The data from two subjects has been utilized for blood volume analysis only. In the case of Subject A, the English saddle was not available at the time of his study and tilt studies were performed with the subject resting on his feet. Data from Subject B has been similarly analyzed. His hemodynamic data was incomplete as the catheterization procedure was terminated when atrial fibrillation developed during catheter placement after bedrest. This persisted for four hours, terminating shortly after intravenous injection of 0.5 mgm of digoxin. The data from an additional subject (C) is excluded from the present analysis as he was found to have chronic hepatitis during the course of the study. Three additional subjects began the study.

One (X) was terminated during the first catheterization procedure because of the development of atrioventricular block and also because of equipment failure. A second (Y) was terminated on the ninth day of bedrest when he was found using the sitting position for bowel action. This appeared to be a situation of misunderstanding but deliberate disruption of bedrest could not be excluded. A third (Z) volunteer was terminated during preparation for the pre-bedrest cardiac catheterization when he manifested evidence of excessive anxiety. Thus, the bulk of the data presented in this document concerns twenty subjects (1-20), with certain data being taken from an additional two subjects (A and B).

Certain similarities between the manifestations of post-recumbency orthostatism and idiopathic orthostatic hypotension plus the excellent therapeutic results obtained with 9-alpha-fluorohydrocortisone in the latter, suggested that this drug might provide valuable prophylaxis from post-recumbency orthostatism. A dosage was selected for use in the present study which would result in sodium and water retention and plasma volume maintenance, but which, hopefully, would not result in recumbent hypertension. This dosage, 0.1 mgm twice daily, was given to eleven subjects during the period of bedrest. The remaining subjects were untreated.

B. Detailed Protocol

1. Daily Procedures Consisted Of:

a) Temperature, pulse, respiration and blood pressure recording, q.i.d.

b) Determination of body weight to 5 gram accuracy. This was measured on a metabolic balance immediately after completion of urine collection at 7:30 A.M.

c) Subjects were required to perform 50-100 watt bicycle exercise twice daily for 10 minutes during the ambulatory phase. This was done in order to make hospital activity correspond more closely with their normal activity.

2. Urine Collection and Analysis:

Urine was collected on a twenty-four hour basis, from 7:30 A.M. to 7:30 A.M., on the initial group of subjects (A,B,C, 1-11). On the remaining subjects (12-20) urine was collected for forty-eight hours, except on odd days (e.g., Day 7 of the Ambulatory Phase) when single twenty-four hour samples were collected. Each voided sample was placed in a gallon container containing the indicated preservative. The urinal was rinsed with 50 cc of distilled water. This aliquot was also poured into the container. The sum of the rinsing volumes was deducted from the total twenty-four hour volume to determine urine output. Total volume was utilized in calculating balance data.

Pooling of two day samples was done by combining 20% volume aliquots of the two samples into a single sample. (For example: If Day 1 urine volume was 2000 cc, a 20% aliquot or 400 cc was removed; if Day 2 urine volume was 2500 cc, a 20% aliquot or 500 cc was removed; the 400 and 500 cc aliquots were

combined to give a representative 48 hour sample).

On completion of twenty-four hour urine collections, specimens were analyzed as follows:

a) Na^{+}, K^{+}, Cl^{-}, $PO_4^{=}$, Ca^{++}, Mg^{++} osmolality, creatinine. Samples for nitrogen analysis are stored.

b) Aldosterone excretion was determined on selected samples from Days 1 & 2 of Ambulant Phase and Days 1,2,8,9,14 of Bedrest Phase.

c) 17-hydroxycorticosteroids were determined during the Ambulatory Phase on Days 1 & 2 and during the Bedrest Phase on Days 1,2,8,9,14.

d) Urinary catecholamines were determined during the Ambulatory Phase on Days 4,5,7 and during the Bedrest Phase on Days 4,5,11,12.

Toluene was used as a preservative for routine analyses, 6N H_2SO_4 for catecholamine preservation and no preservative other than refrigeration and subsequent freezing was used on samples for aldosterone and 17-hydroxycorticosteroid determinations.

On days when other procedures required determinations on interval samples, the individual samples were analyzed and the twenty-four hour output determined by summation.

In the case of those subjects on whom forty-eight hour urines were collected, specimens were analyzed as follows:

a) All specimens analyzed as noted under twenty-four hour urines (a).

b) Aldosterone was determined on selected samples from Days 1 & 2 of the Ambulatory Phase and on Days 1,2,7,8,13 & 14 of the Bedrest Phase.

c) 17-hydroxycorticosteroids were determined on Days 5 & 6 of the Ambulatory Phase and Days 5,6,11 & 12 of the Bedrest Phase.

d) Urinary catecholamines were determined on Days 3,4, & 7 of the Ambulatory Phase and Days 3,4, 9,10,15 of the Bedrest Phase.

3. Stool Collection and Analysis:

On the first day of both the Ambulatory Phase and the Bedrest Phase and on the morning of the 15th day of the Bedrest Phase, the subject ingested a carmine label. Stools were collected in 3-4 day pools for the Ambulatory Phase beginning with the appearance of the first label. On the appearance of the second label, stool collections for the Bedrest Phase were started, again in 3-4 day pools. Stools were analyzed for Na^{+}, K^{+}, Ca^{++}, Mg^{++}. Aliquots are stored for nitrogen analysis.

4. Blood Work

a) RISA-I^{131} Plasma Volume was determined during the Ambulatory Phase on Day 1 & 7. This study was repeated during the first hour after assuming bedrest on Day 1 of the Bedrest Phase, and again on Days 2,6,12 and 15 of the Bedrest Phase.

Methodology used in performing the plasma volume studies was as follows:

1) A sample of blood was obtained for background counting.

2) A known radioactive dose of RISA-I^{131} was delivered intravenously. The syringe was capped for residual counting.

3) Blood samples were obtained at 10,20, & 30 minutes for measurement of I^{131} concentration.

b) C.B.C., E.S.R., serum proteins, creatinine, osmolality, $Na,^{+}$ $K,^{+}$ $Cl,^{-}$ $PO_4,^{--}$ $Ca,^{++}$ $Mg,^{++}$ cholesterol and triglycerides were determined on Day 1 of both the Ambulatory and Bedrest Phases and on Day 12 of bedrest.

c) Additional samples were drawn in relation to renal clearance studies and cardiac catheterization procedures. These will be noted under the appropriate heading.

5. Renal Clearance Studies

These studies were performed on Subjects A,B, & 1-9 on Day 6 of the Ambulatory Phase and Day 6 & 12 of the Bedrest Phase. This procedure was carried out as follows:

a) Blank plasma and urine samples were obtained.

b) A loading dose of paraminohippurate and inulin was infused. This dose was calculated as follows: P.A.H. Loading Dose = 0.008 gm/kg body weight x body weight (kg).

Inulin Loading Dose = 0.05 gm/kg body weight x body weight (kg).

c) A constant infusion of a solution containing paraminohippurate and inulin was delivered at a rate of 2 cc/minute. The concentration of solution used was calculated as follows:

Inulin Sustaining Dose (mgm/ml) =

$$\frac{C_I \times \frac{\text{B.S.A. } (M^2)}{1.73\ M^2} \times U_c}{2 \text{ cc/min.}}$$

where C_I is the predicted normal clearance of inulin by a normal man with a body surface area of 1.73 M^2 (CI = 125 cc/min), B.S.A. is the subjects body surface area, U_c the desired concentration of inulin in the urine, and 2 cc/min. the rate of intravenous infusion.

PAH Sustaining Dose (mgm/ml) =

$$\frac{C_{PAH} \times \frac{\text{B.S.A. } (M^2)}{1.73\ M^2} \times U_{PAH}}{2 \text{ cc/min.}}$$

where C_{PAH} is the predicted normal clearance of PAH by a normal man with a body surface area of 1.73 M^2 (C_{PAH} = 650 cc/min), B.S.A. is the subjects body surface area, U_{PAH} the desired urine concentration and 2 cc/min the rate of intravenous infusion.

d) After allowing 45 minutes for equilibration, the patient voided. This sample was discarded.

e) Two thirty-minute clearance periods were studied. Urine was collected at the end of each. A blood sample was obtained at the midpoint of each period.

f) Plasma and urine samples were analyzed for:

1) PAH

2) Inulin

3) Creatinine

4) $Na,^{+}$ $K,^{+}$ $Cl,^{-}$ $PO_4,^{--}$ $Ca,^{++}$ $Mg.^{++}$

This study was discontinued after subject 9 because of erratic results due to incomplete bladder emptying. This was particularly noticable during the Bedrest Phase.

6. 70° Tilt Without Vascular Instrumentation:

All subjects were subjected to a 70° passive tilt during the Ambulatory Phase. This tilt was performed with a minimum of instrumentation (EKG, tachometer, and mannual blood pressure cuff). The tilt was maintained for twenty-minutes in all cases. This served as a final evaluation to exclude subjects with autonomic insufficiency from the study.

7. Cardiac Catheterization Protocol:

These procedures were carried out in an identical manner on Day 7 of the Ambulatory Phase and Day 15 of the Bedrest Phase.

The subject arrived at the cardiac catheterization laboratory in a fasting and unpremedicated state. He was instrumented for recording of EKG, phonocardiogram and impedance pneumograph. The EKG was coupled to an R-R interval tachometer for recording beat-to-beat heart rate. One forearm was prepared and an 18G Cournand needle introduced into the brachial artery under xylocaine anesthesia. The opposite arm was prepared and draped. Using xylocaine anesthesia a small incision was used to isolate a branch of the median basilic vein. A No. 6 Goodale-Lubin woven dacron catheter was advanced from this vein into the main pulmonary artery utilizing fluoroscopic image amplification.

P23D Statham transducers were utilized in measuring pressures directly from the brachial and pulmonary arteries. These were zeroed at the level of the phlebostatic axis. All parameters were recorded on an eight-channel oscilloscopic recorder.*

a) Baseline Procedures:

Brachial and pulmonary arterial pressures were recorded before each of two collections of expired air for determination of oxygen consumption. The actual collection period followed several tissot "wash-out" collections used to insure stability of respiratory minute volume and to fill dead-space with subject's expired air. The collection periods were two minutes each. At the mid-point of each collection, cardiac output was determined by the indicator-dilution technique utilizing indocyanine green dye. The performance of this technique required

*Electronics for Medicine, Model DR-8.

continuous withdrawal of blood from the arterial needle through a minimum dead space of polyethylene tubing and thence through a densitometer* cuvette at a rate of approximately 30 cc/minute. The densitometer output was fed to the oscilloscopic recorder. Simultaneous with delivery of a known quantity of dye into the pulmonary artery as a single bolus, the tracing of the densitometer was marked by a millivolt signal. Withdrawal of blood was continued until the complete dye dilution curve was visualized on the oscilloscope.

b) 70° Passive Tilt:

Following completion of the above baseline studies, the English saddle was bolted to the fluoroscopy table and the transducers were re-zeroed to a level previously determined to be that of the volunteer's phlebostatic axis in the 70° tilt position.

With the recorder running, the motor of the fluoroscopy table was activated. The time from onset of tilting to achievement of the 70° tilt position was recorded by a millivolt signal and required 10-15 seconds. The location of the 70° axis was determined by a bubble-level affixed to the fluoroscopy table.

On arrival at the 70° axis, pressure transducers were connected to the brachial artery needle and pulmonary artery catheter. Recording was continuous until termination of the tilt procedure. A paper speed of 5 mm/sec. with time lines at 1.0 sec. was used for continuous recording. At one minute intervals paper

*Gilford Model 103(IR)

speed was increased to 25 mm/sec. with time lines at 0.1 sec. for a period of 5-10 seconds. The only interruptions in pressure recordings were those required for flushing to eliminate damping of the pressure curve and those required for cardiac output determinations. The latter were performed at 5 & 18 minutes of tilt in all cases able to tolerate tilting for those intervals. In subjects 9 through 20, cardiac output was also determined after 10 minutes of tilt.

Termination of tilt was scheduled after 20 minutes in stable subjects. However, tilt was immediately terminated at the onset of pre-syncopal symptoms or on a definite drop in arterial blood pressure below 90 mm Hg. systolic pressure. Tilt-down was noted by a millivolt signal.

On return to the horizontal position, transducers were rapidly rezeroed to the original supine phlebostatic axis, and pressure recording was resumed. Monitoring was continuous for 5 minutes, with a spot recording at 10 minutes. Subjects who had not stabilized at 10 minutes were monitored until stability was achieved.

c) Bicycle Ergometric Studies:

This study was performed after pressures and heart rate had returned to basal levels. The exercise was performed by the subject while lying on the fluoroscopy table. The bicycle ergometer was firmly attached to the foot of the table.

The initial study consisted of pedaling at a work-load of 30 watts* (subject 1,2,3) or 50 watts (all other subjects). A steady state was assumed to have been reached after four minutes of exercise. At this time pressures were recorded and a two minute collection of expired air was begun. At the mid-point of this collection, cardiac output was measured as previously described. On completion of expired air collection, exercise was stopped and the subject allowed to return to his previous basal state.

Following the initial exercise period, during which cardiac output was measured, step-wise increases in work-load were induced. In subjects 1 to 11 work-load was increased without intermediate rest periods from 50 watts to a maximum of 200 watts in 25 watt increments. Exercise was discontinued when heart rate reached 180/minute at any step. Recording was continuous during the initial five minutes of the recovery period with further spot recordings at five minute intervals until basal levels were reached.

In subjects 12 to 20 procedures were refined to include the achievement of the steady state at each exercise step, with measurement of pressures and heart rate at the 4th and 6th minute and expired air collection from the 4th to 6th minute or 4th to 5th minute depending on the exercise level. Sufficient rest was allowed between each step to permit return of heart rate and blood pressure to starting levels. The exercise period during which the subject reached a heart rate of 180/minute or a systolic

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*watt = 6.12 kgM/min.

arterial pressure of 200 mm Hg was considered the final exercise level. On cessation of exercise, recovery was followed as in earlier studies.

d) Tyramine Stimulation Test:

This portion of the catheterization protocol was designed to yield information on the role of deranged sympathetic nervous function in post-recumbency orthostatism. Tyramine is known to result in liberation of the loosely-bound norepinephrine stores from nerve endings (124,125,169). In the normal individual, norepinephrine release would be expected to result in a blood pressure rise. In the presence of normal norepinephrine stores and malfunctioning receptors, norepinephrine release would not produce a blood pressure rise, but might produce a rise in plasma norepinephrine. If stores were depleted, neither blood pressure or plasma norepinephrine would be expected to rise.

Based on this hypothesis, 3 mgm of tyramine* was injected into the pulmonary artery with continuous pressure monitoring for a period of 5 minutes with an additional spot check at 10 minutes. Prior to the injection and 5 minutes after injection, a blood sample was obtained for subsequent norepinephrine determination. Each patient was requested to empty his bladder prior to the tyramine injection. Two one-hour urine samples were collected after the injection.

e) Valsalva Maneuver:

Subjects 16-20 were required to perform a controlled

*Obtained in sterile 1 mgm/cc ampoules from Merck, Sharp & Dohme.

Valsalva maneuver prior to tilting during catheterization study pre- and post-bedrest. These were performed by having a patient blow into a Flack tester device obtained from NASA's Bioinstrumentation Section. This was a whistle-like device with a spring loaded plunger providing a 40 mm Hg resistance to expiration. The subject maintained this forced expiration for a period of 10 seconds. Brachial and pulmonary artery pressures, EKG and heart rate were continuously monitored.

III. BIOCHEMICAL METHODS

All studies were performed in duplicate.

A. Sodium & Potassium

1. Reagents

   a) 1% Sterox (Harleco)

2. Standards

   a) Stock Standard (Harleco) Na^+ 145 mEq/L, K^+ 4.5 mEq/L.

   b) Working Standards - The stock standard is utilized for the upper levels. A lower level is determined by diluting stock solution so that it contains Na^+ 118 mEq/L and K^+ 3.8 mEq/L.

3. Serum Sodium & Potassium Determinations

   a) Serum is diluted 1:100 in 0.02% Sterox as follows:

      1) 5 cc of distilled water is added to a 10 cc volumetric flask.

      2) 0.2 cc of 1% Sterox solution is added slowly to avoid foaming.

      3) 0.1 cc of serum is added.

      4) Flask is filled to mark with distilled water.

   b) A Junior Coleman flame spectrophotometer is zeroed with 0.02% Sterox.

   c) Samples are read, using the working standards above.

4. Urine Sodium & Potassium Determinations

   a) A 1:100 initial dilution of urine is used. For

potassium additional dilution to 1:500 is necessary.

b) Procedure is otherwise the same as for serum.

B. Calcium & Magnesium

1. Reagents

a) 5% Trichloracetic Acid - 1% Lanthanum Chloride diluting fluid.

2. Standards

a) Stock Standard - A 50 mEq/L stock solution is made by dissolving pure magnesium metal turnings in an appropriate volume of concentrated hydrochloric acid. This is diluted to volume with double distilled water.

b) Working Standards - Stock standard is diluted in 5% TCA - 1% $LaCl_2$ to make magnesium standards of 0.025, 0.050, 0.075, 0.10, and 0.125 mEq/L, and calcium standards of 0.10, 0.16, 0.20, 0.24 and 0.26 mEq/L.

3. Serum Calcium & Magnesium Determinations

a) Serum is diluted 1:25 in the 5% TCA-1% $LaCl_2$ diluting fluid. This is centrifuged and the supernatent compared with standards on a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer, utilizing the appropriate calcium or magnesium hollow cathode lamp.

4. Urine Calcium & Magnesium Determinations

a) An initial 1:25 dilution of urine in the 5% TCA-1% $LaCl_2$ diluting fluid is run. Subsequent appropriate dilutions are made.

C. Inorganic Phosphorus

1. Reagents

a) 10% Ammonium Molybdate; 50 gms. of dry ammonium molybdate are dissolved in 500 cc of 10N H_2SO_4.

b) Ferrous Sulfate Molybdate; 5.0 gms. of ferrous sulfate are dissolved in 50 cc of distilled water. 10 cc of 10% ammonium molybdate are added and the volume brought to 100 cc with distilled water. Color should be dirty grey. Must make reagent fresh each run.

c) 12% Trichloroacetic Acid; 12 gms. of TCA in 100 cc distilled water; filter and refrigerate.

d) 34% Trichloracetic Acid; 34 gms. of TCA in 100 cc distilled water; filter and refrigerate.

2. Standards

a) Potassium Acid Phosphate - Dissolve 0.438 gm of KH_2PO_4 in 1000 cc of distilled water. This in the stock standard of 100 µgm phosphorus/cc.

b) Working Standards

1) 2 µgm/cc; 2 cc of stock q.s. 100 cc with distilled water.

2) 4 µgm/cc; 4 cc of stock diluted to 100 cc.

3) 8 µgm/cc; 8 cc of stock diluted to 100 cc.

3. Serum Phosphorus Determination

a) To 0.2 cc of serum slowly add 3.5 cc of 12% TCA. Allow to stand 10 minutes, then centrifuge for 10 minutes. Separate supernatant.

b) Set up tubes as follows:

1) Reagent blank; 3 cc 12% TCA

2) Standards; 3 cc 2 µgm/cc

3 cc 4 µgm/cc

3 cc 8 µgm/cc

3) Sample; 3 cc of supernatant

c) Add 2 cc ammonium molybdate ferrous sulfate reagent to all tubes. Let stand 2 minutes. Read at 690 mµ. Reagent blank is used for zero. Tubes are stable for 2 hours.

d) Construct standard curve from standard results. Read unknown result in µgm/cc of filtrate. Multiply by 17.5 (dilution factor)to get µgm/cc of serum. Multiply by $\frac{100 \text{ cc}}{1000 \text{ µgm/mgm}}$ (or divide by 10) to obtain mgm %.

4. Urine Phosphorus Determinations

a) Urine sample is diluted 1:100.

b) Set up tubes as follows:

1) Reagent blank; 2 cc distilled water

2) Standares; as for serum.

3) Sample; 2 cc of 1:100 urine dilution

c) Add 1 cc of 34% trichloracetic acid to all tubes.

d) Add 2 cc of freshly prepared ammonium molybdate ferrous sulfate solution to all tubes.

e) Let tubes stand for at least 2 minutes (Color stable 2 hours).

f) Read as for serum.

D. Chlorides - Determined by use of Buchler-Cotlove Chloridometer.

E. Osmolality - Determined on a Fiske Osmometer, Model G, early in the study; then on an Advanced Instruments Osmometer.

F. Creatinine

1. Reagents:

a) 0.04 N Picric Acid; 10.1 gm. picric acid diluted to 1000 cc with distilled water. Slight sediment will appear; filter or let settle.

b) 0.75 N NaOH; 30 gm NaOH diluted to 1000 cc in distilled water.

2. Standards

a) Stock Creatinine (1 mgm/cc) - Dissolve 1.0 gm creatinine in 1000 cc 10N HCl. Keep refrigerated in dark bottle.

b) Working Standards

1) 0.001 mgm/cc; 0.1 cc of stock q.s. distilled water to 100 cc.

2) 0.003 mgm/cc; 0.3 cc of stock q.s. distilled water to 100 cc.

3) 0.005 mgm/cc; 0.5 cc of stock q.s. distilled water to 100 cc.

4) 0.010 mgm/cc; 1.0 cc of stock q.s. distilled water to 100 cc.

Working standards must be freshly made.

3. Serum Determination

a) Preparation of filtrate (Folin Wu)

1.0 cc serum)
7.0 cc distilled water)
1.0 cc 2/3 N H_2SO_4) = 1:10 dilution
1.0 cc 10% Na Tungstate)

b) Mix and centrifuge 10 minutes.

c) Colorimetry

1) Set up tubes as follows:

Reagent blank		3 cc distilled water
Standard	(0.001 mgm/cc)	3 cc
	(0.003 mgm/cc)	3 cc
	(0.005 mgm/cc)	3 cc
	(0.010 mgm/cc)	3 cc
Sample		3 cc Folin Wu filtrate

2) Add 1 cc of 0.04 N Picric Acid to all tubes.

3) Add 1 cc of 0.75 N NaOH to all tubes.

4) Mix and allow to sit for 20 minutes after the addition of NaOH.

5) Read at 525 mμ using reagent blank as zero.

d) Calculation - Read mgm/cc of filtrate from the standard curve. Multiply by 10 to determine mgm/cc of serum. Multiply by 100 to obtain mgm %.

4. Urine Determinations

a) Urine is diluted 1:100.

b) 3 cc of this dilution is treated the same as the Folin Wu filtrate.

G. Urine Creatinine

Discontinued after method proved to be inadequate after first few subjects.

H. Serum Triglyceride

1. Reagents

a) Silicic Acid, 100-200 mesh Unisil

Source: Clarkson Chemical Co., Inc. Williamsport, Pa.

b) Ethanol - KOH - Stock: 2.5% KOH aqueous

Working: 0.025% alcoholic KOH. Make up fresh every 2 weeks by diluting stock with 95% ethanol.

c) 0.7 M H_2SO_4

d) Hexane

e) Sodium Periodate 0.02 N - Stock should be sealed in small vials as decomposition occurs spontaneously (Yellow color indicates decomposition). 1.15 gm periodic acid dissolved in 225 ml of distilled H_2O. Neutralize with 0.2 N NaOH to methyl red endpoint. Do not over-neutralize; decomposition

will occur and a yellowed solution result.

Source: Periodic Acid Crystals, one ounce bottle. Matheson, Coleman and Bell, Norwood (Cincinatti) Ohio, 887 P x 400.

f) Sodium Arsenite 0.2 N - Replenish every two months to avoid a rising blank.

1.8 gm NaOH and 4 gm Arsenious Acid dissolved in 200 ml distilled H_2O.

Source: Baker & Adamson, 1 lb. bottle P 183. Code 1037 Arsenious Acid (As_2O_3), General Chemical Division, Allied Chemical & Dye Corp., New York.

g) Chromotropic Acid Reagent

Slowly mix 300 ml concentrated H_2SO_4 (C.P.) with 150 ml distilled H_2O in a Pyrex Erlenmeyer in an ice bath. Dissolve 1 gm Chromotropic Acid (Disodium salt) in 100 ml distilled H_2O, filter and stir into the diluted acid after it has been cooled. Store in a dark bottle. Replenish each week.

Source: 4,5 dihydroxy - 2,7 naphthalenedisulfonic acid disodium salt T 230, 250 gm bottle (Technical Grade), Eastman Organic Chemicals, Distillation Products Industries, Rochester 3, New York.

h) Methanol, Reagent Grade

i) Chloroform, Reagent Grade

2. Standard

   a) Stock: Tripalmitin in Chloroform 5 mg/ml.

   b) Working Standard: Dilute 0.5 ml of stock to 25 mm.

3. Triglyceride Determination

   a) Extraction: Pipette 0.5 ml of serum or plasma into a 125 ml screwcap bottle. With mixing add 5 ml MeOH and 10 ml $CHCl_3$, mix well. Add 15 ml acidulated H_2O, do not mix; allow to stand overnight. (Acidulated H_2O prepared by adding 1 drop 50% HCl to each 10 ml distilled H_2O). Remove $CHCl_3$ layer with syringe and canula to 15 ml stoppered centrifuge tube and add about 0.5 gm Silicic Acid. Mix (Vortex) and let stand 20 minutes. Centrifuge for 10-20 minutes at 2000 RPM. Transfer supernatant to another tube and take 0.5 ml aliquots from there. Pipette 200 λ, 500 λ, and 1000 λ of the working Tripalmitin standard in duplicate into a 15 ml stoppered tube. All tubes evaporate to dryness. Include a blank tube in each run.

   b) Hydrolysis of triglycerides: To all tubes add 0.2 ml of 0.025% alcoholic KOH and hydrolyze for 30 minutes at 60°C. Cool.

   c) Extraction of Fatty Acids: Add 0.1 ml 0.7 M H_2SO_4 and 4 ml Hexane, stopper; shake vigorously

for a few seconds, then suction away and discard the hexane phase (Set in 60° C bath for a few seconds to remove last traces of hexane).

d) Oxidation of Glycerol: Add 0.1 ml and 0.02 N Na periodate, mix by tapping, and allow to stand 10 minutes.

e) Reduction: Add 0.1 ml 0.2 N Na Arsenite, mix, and allow to stand 5 minutes.

f) Color Development: Avoiding direct light, add 4 ml Chromotropic Acid solution; buzz and capp with aluminum foil. Place in boiling bath for 30 minutes. Cool and read in Junior Coleman at 570 mμ.

g) Calculation:

$$\gamma \text{ obtained from standard curve} \times \frac{10}{0.5} \times 2 \times \frac{100}{1000} = \gamma \times 4 =$$

mgm% triglycerides.

I. Total Serum Cholesterol

1. Extraction

a) Reagents

1) Alcohol-ether mixture (made fresh daily).

2) 3 volumes of Ethanol, absolute, reagent grade.

3) 1 volume Ether (Squibb for anesthesia).

b) Procedure

Bring vials of serum to room temperature. Pipette

0.5 ml serum to 25 ml volumetric flask to which 7 ml alcohol-ether (3:1) mixture has already been added. Add serum slowly and swirl flask as the serum is added. Set aside 30-60 minutes. Bring contents of flask to a boil in 100° oil bath. Mix while heating. Allow the extract to cool. Bring to volume with the alcohol-ether mixture. Place stoppers in flask and mix well. Filter immediately through Whatman #541 paper; collect in 20 x 150 screw cap tube; cap immediately to prevent evaporation. This gives a 1:50 dilution of the serum.

2. Colorimetric Determinations

a) Reagents

1) Stock dibromide purified cholesterol standard- 250 µgm/cc prepared fresh every two weeks, made up in absolute alcohol. Store in stoppered reagent bottle at room temperature.

2) Acetic acid - glacial acetic acid.

3) $FeCl_3 \cdot 6H_2O$ solution - made fresh on day of analysis. 5 mg $FeCl_3$ and 4 ml acetic acid per sample.

4) Concentrated H_2SO_4 , Merck, 1 lb. bottle, specific gravity 1.826 - 1.838.

b) Procedure

Place 1 ml aliquot of unknown extract and 1 ml aliquot of all 5 standards in screw cap tube and evaporate to dryness in a vacuum manifold apparatus. Two blanks-1 ml alcohol-ether mixture and 1 ml alcohol, evaporated to dryness. All tuces flushed with nitrogen. Add 4 ml acetic acid-ferric chloride solution to each tube and mix to dissolve the residue. Let stand while standards are being prepared.

3. Standards

Working Standards

a) 0.5 ml stock diluted to 10 ml with absolute alcohol. 1 ml = 12.5 micorgrams.

b) 1 ml stock diluted to 10 ml with absolute alcohol. 1 ml = 25 micrograms.

c) 2 ml stock diluted to 10 ml with absolute alcohol. 1 ml = 50 micrograms.

d) 3 ml stock diluted to 10 ml with absolute alcohol. 1 ml = 75 micrograms.

e) 4 ml stock diluted to 10 ml with absolute alcohol. 1 ml = 100 micrograms.

Working standards prepared fresh on the day of analysis.

4. Unknowns, standards and blanks are carried through

remainder of the procedure in random, blind order--i.e. analyst cannot identify any sample.

Heat water bath to 56°, prepare ice bath in 1 liter beaker. Set timer and begin timing the following steps as described. When timing begins, tubes are placed, one at a time at 1/2 minute intervals, in the ice bath, beginning with the blank tubes. Each tube remains 1-1/2 minutes in the ice bath (cooled 1 minute before adding H_2SO_4 and 1/2 minute afterward). After 1 minute in the ice bath, 3 ml of H_2SO_4 are forced into each tube; the tube is capped, contents stirred and placed in 56° water bath. As each tube is taken out of the ice bath, another is placed in the ice bath to take its place so that there are 3 tubes in the ice bath at the same time. Each tube is left in the 56° bath 5 minutes.

When tubes have been removed from the 56° bath, contents are transferred to Coleman tubes for reading on a Coleman Senior Spectrophotometer. Reading time begins one hour from the time the first tube is placed in the 56° bath and continues at precisely 1/2 minute intervals. Read at 555 wave length and record optical density. A standard curve is prepared (or its slope is calculated) and concentrations for unknowns are determined from the curve.

J. Serum Proteins

1. Reagents

a) 26% Na_2SO_3 (Keep at 37°C at all times).

b) Biuret reagent

c) Ether

2. Total Protein

a) Set up centrifuge tubes as follows:

Unknown	Blank	
4.9 cc	5.0 cc	Distilled water
0.1 cc		Serum
3.0 cc	3.0 cc	Biuret reagent

b) Mix by swirling. Allow to stand for 30 minutes.

Read at 540 mμ. Read gm% from standard curve.

3. Albumin

a) Set up a centrifuge tube as follows:

4.8 cc - 26% Na_2SO_3

0.2 cc - Serum

1 inch - Ether

b) Shake vigorously for 30 seconds.

c) Centrifuge for 10 minutes.

d) Draw off globulin-ether layer.

e) Set up test as follows:

Unknown	Blank
2.5 cc Filtrate	2.5 cc Distilled water
2.5 cc Distilled water	2.5 cc Na_2SO_3
3.0 cc Biuret reagent	3.0 cc Biuret reagent

f) Let stand for 30 minutes.

g) Read at 540 mμ.

h) Determine gm% from standard curve.

K. Urine Nitrogen - Kjeldahl Method by Autoanalyzer

Autoanalyzer method inadequate. Awaiting determination.

L. Urine 17-Hydroxycorticosteroid - Performed by Porter-Silber Method.

M. Urine Aldosterone - Method of Kleiman & Peterson, J. Biol. Chem. 235: 1639, 1960.

N. Inulin

1. Reagents

a) 0.6% indolacetic acid ethyl ester (Calbiochem, Grade A) in absolute alcohol. Stable in refrigerated brown bottle for several weeks.

b) Concentrated Hydrochloric acid.

c) Cadmium sulfate solution

1) 34.67 grams - $3\ CdSO_4 \cdot 8\ H_2O$

2) 16.9 ml - 1 N H_2SO_4

3) Distilled water q.s. to 1000 ml.

d) 0.5 N NaOH

2. Standard

a) Stock 0.1% Inulin

b) Working standard

1) Serum 0.01, 0.03, 0.06 mgm/ml

2) Urine 0.01, 0.02, 0105 mgm/ml

3. Determination

a) $CdSO_4$ filtrate

0.5 ml of plasma or urine

0.5 ml H_2O

3.0 ml Cadmium sulfate solution (1d)

1.0 ml 0.5 N (approximate) NaOH (12.5 gm q.s. 500 cc)*

*Note: 3 ml of $CdSO_4$ solution and 1.0 ml of 0.5 N NaOH should be pre-checked. If this mix produces pink reaction with phenopthalein, then pH is acceptable.

b) Dilute urine filtrate 1:10.

c) Add 0.1 ml of indolacetic acid ethyl ester reagent (1a) to 0.5 ml of filtrate, mix. Add 4.0 ml of concentrated HCl, mix.

d) Allow to stand at room temperature overnight (cap tube with a marble).

e) Read at 530 mμ.

O. Para-aminohippurate

1. Reagents

a) 1.2 N HCl

b) 100 mgm% $NaNO_2$ (prepare fresh every 3 days).

c) 500 mgm% Ammonium sulfamate (stable 2 weeks).

d) 100 mgm% N (1 naphtyl)-ethylene-diamine dihydrochloride. (Refrigerate in brown bottle).

e) Cadmium sulfate solution (See inulin method).

f) 0.5 N NaOH.

2. Standard

a) Stock 0.02% PAH

b) Working

1) Serum 0.001, 0.002, 0.004 mgm/ml

2) Urine 0.008, 0.0012, 0.0016, 0.002 mgm/ml

3. Determination

a) $CdSO_4$ filtrate -- see inulin method.

b) Dilute urine filtrate 1:100.

c) Add 0.4 ml of reagent (1a) to 2 ml of filtrate or diluted filtrate, mix.

d) Add 0.2 ml of reagent (1b), mix.

e) Add 0.2 ml of reagent (1c), mix.

f) Add 0.2 ml of reagent (1d), mix.

g) Read at 540 mμ 10 minutes after adding reagent (1d).

P. Plasma Catecholamines (Method of H. Weil-Malherbe)

1. Reagents

a) Alumina (Al_2O_3) - Woelm, neutral, activity grade 1 (Alupharm Chemicals, New Orleans). To 100 gms add 50 cc of 2 N HCl, bring to 80°C, stirring constantly and rapidly for 20 minutes. Filter through a Buchner funnel and wash with 500 cc of hot 2 N HCl. Rewash with 500 cc aliquots triple distilled water about 15-20 times. Stir during wash. Filter through a Buchner funnel. Dry at 300°C for 3 hours. Keep dry at all times at a temperature of approximately 45°C.

b) Disodium ethylenediaminotetracetate (EDTA) 1% (w/v).

c) Amberlite 1 RC-50 (or CG-50), type 2.

Soak resin in 2 N HCl for 24 hours. Wash by decanting. Soak in H_2O for 24 hours. Decant. Soak in 1 N NaOH for 24 hours. Decant. The resin is stirred for 30 minutes in each solution. Repeat this cycling twice. 1 M sodium acetate, pH 6.0, for 30 minutes. Adjust the pH of the mixture to 6.0 with acetic acid. Leave in 1 M sodium acetate, pH 6.0, for 12 hours. Adjust the pH to 6.0 over a period of 2 hours. Store the resin in acetate, buffer pH 6.0. Before use, wash the required amount with H_2O and suction free of adhering moisture.

d) Ethylenediamine

Redistill the commercial product in batches of 200 ml in an all-glass apparatus, rejecting the initial and final portions of the distillate. This reagent is sufficiently stable for several weeks when stored in a dark, glass-stoppered bottle in the refrigerator.

e) Isobutanol

Reflux commercial product over NaOH, 20 g/L, for 8 hours, and distill. Follow the same procedure

as for ethylenediamine. Store in a dark, glass-stoppered bottle in the refrigerator.

f) Acetic acid 1.0 M.

g) Acetic acid 0.2 M.

h) Ammonium acetate 1 M.

i) Sodium hydroxide 1 N.

j) Sodium carbonate 0.5 N.

This is treated by passing it through a column of treated 1R-120 C.P. cation exchange resin. The resin is treated in the following manner. Soak in 1 N HCl for 24-48 hours, decant. Wash with H_2O by decantation. Leave in 10% NaCl for approximately 30 minutes with occasional stirring. Wash with H_2O again. Repeat this cycling two to three times. Before use, repeat HCl, NaCl cycling once in a column, then pass 0.5 N sodium carbonate through. The column should not be allowed to run dry.

2. Standards

a) Stock - 50 µg/cc of noradrenalin and adrenalin are kept refrigerated in low actinic flasks. These are made by the following procedure:

Noradrenalin - 9.95 mg of l-noradrenalin bitartrate hydrate are dissolved in q.s. 0.1 N HCl to 100 cc.

Adrenalin - 9.1 mg of l-epinephrine bitartrate are dissolved in q.s. 0.1 N HCl to 100 cc. Both are stable for years if refrigerated in low actinic flasks.

b) Working Standard

For both adrenalin and noradrenalin, dilute 0.1 cc of stock standard to 100 cc with triple distilled water. This gives a concentration of 0.05 μgm/cc. These must be prepared fresh daily.

3. General

a) All water used is double distilled. The last distillation is glass distilled.

b) All glassware is cleaned with lab cleaner, treated with dichromate solution.

4. Sample Handling

a) Thirty to forty cc's of blood are drawn and transferred to a 40 ml glass-stoppered ice-cold centrifuge tube containing 5 drops of heparin. This is mixed.

b) Sample is iced and then centrifuged as soon as possible in a refrigerated centrifuge at approximately 4°C for 20 minutes.

c) Plasma is separated immediately.

d) Sample should be run at once. If not, it should be frozen.

5. Procedure

a) Weight out 0.4 gram of treated Pl_2O_3 and place into a glass-stoppered tube. Add 9 ml. H_2O and 1 ml. 1 M ammonium acetate. Add a sufficient

amount of 1 N NaOH to bring the solution to pH 8.4. The amount of 1 N NaOH added at this point must be predetermined from each treated batch of alumina. Invert tube several times very gently. The alumina, being fragile, should not be handled roughly.

b) Preparation of Columns and Sample

1) The column consists of a 50 ml spheric bulb and a stem of 5 mm bore with a constriction about 15 cm below the bulb. The top of the bulb is fitted with a spheric socket joint, 18/9.

2) Place a small wad of glass wool over the constriction. Fill column with H_2O. Carefully pour Al_2O_3 emulsion into H_2O in column. Allow Al_2O_3 to settle, washing down sides of column with H_2O if necessary. Pressure may be applied to expedite flow.

3) To 8-10 ml of plasma add a sufficient amount of 0.5N Na_2CO_3 to bring pH to 8.4. Constant monitoring on a pH meter is necessary. In order to facilitate flow through the column it is often necessary to dilute the plasma about 5:1 with H_2O. This is done providing the blood has not been previously diluted with anticoagulant.

c) Adsorption and Elution from the Alumina Column

1) After H_2O has passed through the column, remove pressure and add the diluted plasma pH 8.4. The plasma is allowed to flow through the column at a rate of 0.5 to 1.0 cc per minute. If flow is too fast, it may be controlled by inserting a small piece of tygon tubing with Hoffman clamp to bottom of column. If flow is too slow, pressure may be applied. The beaker which contained the plasma is rinsed with 10 cc of H_2O and poured into column after plasma has passed through. This is followed by 2 washings with 8-10 cc of H_2O. Pressure may be applied for all of these washings. After the final washing, the pressure is released, the Hoffman clamp is closed and any water remaining in the lower end of the tygon tubing is shaken out. All that has passed through the column at this point is discarded.

Elution is carried out with 3 ml of 0.2N acetic acid. This is passed through the column slowly (no pressure), and collected in a 10 cc beaker. This is followed by 3 cc of H_2O which is collected in the same beaker.

The last drops of H_2O are blown out of the column into the beaker.

d) Absorption and Elution for the Amberlite Column

1) For this procedure a clean dry column is used. A small wad of glass wool is placed over the constriction.

2) Filter CG-50 resin pH-50 resin pH 6.0 through a Buchner funnel and wash with water allowing all excess liquid to filter out. Weigh out 0.4 mg resin and insert into column bulb.

3) Fill column with H_2O to wash down resin. Pressure may be applied.

4) With 1N NaOH bring acetic acid eluate to pH 6.0.

5) After H_2O has passed through the column add the aortic acid eluate. This must pass through the column relatively slowly taking approximately 30-60 minutes. Because the resin shrinks in on acid medium, fast filtration may occur. This can be prevented by tamping down the column with a glass rod.

6) The 10 cc beaker is rinsed with approximately 10 cc of H_2O and passed through the column after the acetic acid has passed through. Here pressure may be applied. Up to this point all solutions passed through the column are discarded. Again the flow is shut off via

the clamp, and any excess H_2O in the lower portion of the tygon tubing is shaken out.

7) The column is then eluted with 3 cc of 1 M acetic acid. The first 1/2 cc to pass through is collected in a calibrated centrifuge tube and discarded. Another 1/2 cc is collected in a glass stoppered low-actinic tube. The clamp is then closed and the column is stopped for 20 minutes. This is done to allow the resin to reach equilibrium. At the end of 20 minutes, the column is allowed to flow and the remainder of the eluate is collected in the same low actinic test tube. No pressure should be applied during this elution.

e) A column blank is also run with each specimen and set of specimens. It is treated exactly the same as all others, the only exception being that H_2O replaces plasma.

f) It is recommended that no more than eight columns be run simultaneously.

g) Important Considerations

1) The columns should never be allowed to run dry except after the final elutions. If this does occur, it is necessary to prepare new columns.

2) Gas bubbles should never be present in the column.

3) The plasma and eluting solutions should pass through at a relatively slow rate--0.5 to 1.0 cc per minute is recommended for plasma.

h) Formation of Fluorescent Condensation Products

1) A total of from 5 to 11 tubes are carried through this procedure depending upon the number of plasma samples run. Here only one plasma sample is considered. The tubes are numbered and should contain:

#1) 2.5 cc 1M acetic acid - reagent blank

#2) 2.5 cc 1M acetic acid - for norepinephrine standard

#3) 2.5 cc 1M acetic acid - for epinephrine standard

#4) 2.5 cc column blank eluate - column blank

#5) 2.5 cc sample eluate - sample

2) Only low actinic stoppered glass tubes are used.

3) To tube #2 add 0.05 cc of 1µg/cc norepinephrine standard.

4) To tube #3 add 0.05 cc of 1µg/cc epinephrine standard.

5) To all tubes add 0.4 ml redistilled ethylenediamine. The tubes are then stoppered and

shaken vigorously to saturate the solution with air.

6) Place the stoppered tubes in a water bath set at 55°C for 25 minutes.

7) Cool the tubes in running tap H_2O.

8) Add approximately 1.5 gms of NaCl to each tube.

9) Add 1 ml of redistilled isobutanol to each tube.

10) Stopper tubes and shake for 4 minutes on a mechanical shaker.

11) Transfer the upper organic layer (isobutanol) to a microcuvette and read in a spectrophotofluorimeter. The activation beam is set at 420 mμ and readings for each sample are taken with the emission set at 510 and 580 mμ.

i) Readings - The following numbers are provided for illustration purposes only:

Tube	420/510 mμ	420/580 mμ
#1	310	47
#2	1200	189
#3	1050	320
#4	380	71
#5	470	83

Correcting for Blank Readings

#2	1200-310=890	189-47=142
#3	1050-310-740	320-47=273
#4	470-380-90	83-71=12

j) Calculations

1) Let m = fluorescence ration of epinephrine/ norepinephrine standards at 580 mμ.

2) Let n = fluorescence ratio of epinephrine/ norepinephrine standards at 510 mμ.

3) Let A = amount of epinephrine in unknown.

4) Let N = amount of norepinephrine in unknown.

5) Let y = amount of epinephrine corresponding to fluorescence measured at 580 mμ.

6) Let b = amount of epinephrine corresponding to fluorescence measured at 510 mμ.

7) The following 2 equations are set up:

$A + N/m = y$ (Equation I)

$A + N/n = b$ (Equation II)

8) Therefore:

$$N = \frac{mn\,(b-y)}{m-n} \quad \text{(Equation III)}$$

$$A = y - Nm = b - \frac{N}{n} \quad \text{(Equation IV)}$$

9) $m = \frac{273}{142} = 1.92 \qquad n = \frac{740}{860} = 0.831$

10) To determine the amount of epinephrine corresponding to fluorescence at 510 mμ a simple proportion is set up:

$$\frac{740}{.05} = \frac{90}{b}$$

b = 0.0060 μgE fluorescing at 510 mμ.

11) To determine the amount of epinephrine corresponding to fluorescence at 580 mμ a simple proportion is set up:

$$\frac{273}{.05} = \frac{12}{y}$$

y = .0021 μgE fluorescing at 580 mμ.

12) To measure amounts of norepinephrine in plasma, the following substitutions are made in equations III and IV:

$$N = \frac{mn\ (b-y)}{m-n} \quad \text{(Equation III)}$$

$$N = \frac{1.92 \times .831\ (.0060 - .0021)}{(1.92 - .831)}$$

$$= .0056 \times \frac{1000}{\text{amount of plasma used (here 10 cc were used)}}$$

= 0.56 μg N/L of plasma

$$A = b - N/n \quad \text{(Equation IV)}$$

$$A = .0060 - \frac{.0056}{.831} = -.0004$$

No adrenaline is measured = 0.

Note: After extensive work with this method, it was our opinion that the results were too unpredictable. Therefore, analytic activity was discontinued.

Q. Urine Catecholamines

1. Reagents

a) Alumina - See plasma catecholamine method.

b) 0.2 M EDTA - Dissolve 37.2 gm reagent grade disodium ethylenediaminetetracetate by heating in glass distilled water, cool, and make to 500 ml.

c) 5 N NaOH - Dissolve 100 gm NaOH pellets in glass distilled water and q.s. to 500 ml.

d) 0.2 N Acetic Acid - Dilute 5.8 ml glacial acetic acid to 500 ml.

e) 1 M Acetic Acid - Dilute 28.8 ml glacial acetic acid to 500 ml.

f) 1 M Sodium Acetate - Dissolve 68 gm reagent grade sodium acetate in glass-distilled water and dilute to 500 ml.

g) Acetate Buffer, 1 M, pH 6.5 - Adjust 400 ml 1 M sodium acetate to pH 6.5 by the addition of approximately 6 ml of 1 M acetic acid with monitoring on a pH meter.

h) Acetate Buffer, 1 M, pH 3.5 - Adjust 400 ml 1 M acetic acid to pH 3.5 by the addition of approximately 33 ml 1 M sodium acetate with constant monitoring on a pH meter.

i) 0.1 N Iodine Solution - Dissolve 0.635 gm reagent grade Iodine and 2.5 gm reagent grade NaI in 50 ml glass distilled water.

j) 0.05 N Sodium Thiosulfate - Dissolve 2.48 gm reagent grade sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)

in glass distilled water and q.s. to 200 ml.

k) 1% Ascorbic Acid - Dissolve 100 mg of reagent grade ascorbic acid in 10 ml glass distilled water just before use.

l) NaOH - Ascorbic Acid Solution - Mix 7 volumes 5 N NaOH and 3 volumes 1% ascorbic acid immediately before use.

2. Standards

a) Stock

1) 50 µgm/cc as for plasma.

2) 10 µgm/cc - Dilute 10 cc of 50 µgm/cc standard to 50 cc with 0.01 HCl.

b) Working Standards - 1 µgm/cc

Dilute 1 cc of 10 µgm/cc stock standard to 10 ml with glass distilled water.

3. Extraction of Free Catecholamines

a) Measure out 25 cc of urine.

b) Centrifuge urine.

c) Pour off supernatant and to it add 2 cc of 1% ascorbic acid and 2.5 cc of 0.2 M EDTA.

d) Place a layer of glass wool above the stopcock of a chromatographic column.

e) Pour approximately 1 gm alumina in a water slurry into the column.

f) When the alumina has settled add another layer of glass wool.

g) Adjust the pH of the column to pH 8.4 by adding 10 cc of 0.2 M sodium acetate.

h) Just prior to adding the urine aliquot to the column, the urine is adjusted to pH 8.4 by the addition of 5 M NaOH and 0.5 N NaOH. A pH meter is used for this adjustment.

i) The aliquot is added to the column.

j) After the urine has passed through, 20 cc of 0.2 M sodium acetate are passed through the column and discarded.

k) This is followed by 10 cc of glass distilled water which is discarded.

l) Elution is then carried out by the addition of 5 cc of 0.2 N acetic acid. Both the urine and the 0.2 N acetic acid are passed through the column at a rate of 0.5 -/cc/min.

m) The eluate is collected in a 15 ml graduated centrifuge tube.

n) 0.5 cc of 0.2 M EDTA is added to the acetic acid eluate.

o) The eluate is adjusted to 8 cc with 0.2 N acetic acid, mixed by inversion, centrifuged briefly, and transferred to a clean, dry test tube.

p) The eluate is tested for catecholamines immediately or refrigerated - the eluate is stable for at least 2 weeks at 2°C.

4. Formation of trihydroxindoles

a) 14 tubes are set up for each eluate:

Tube No.	Aliqout	Contents
1	0.2 ml NE working standard	NE external standard
2	0.1 ml NE working standard	NE external standard
3	0.2 ml E working standard	E external standard
4	0.1 ml E working standard	E external standard
5	Nothing	Reagent blank
6	0.2 ml eluate	Sample
7	0.2 ml eluate	Sample blank

b) To each tube add 1.0 ml 1 M acetate buffer pH 6.5.

c) To each tube add 0.1 ml 0.1 N iodine solution.

d) Four minutes after addition of iodine add 0.5 ml of 0.05 N sodium thiosulfate - this destroys excess iodine.

e) Mix the 5 N NaOH - 1% ascorbic acid mixture and add 1 ml to all tubes except tube #7.

f) To tube #7 add 0.7 ml of 5 N NaOH, wait 15 minutes to allow any fluorescent indoles formed to deteriorate, then add 0.3 ml 1% ascorbic acid.

g) Dilute all tubes to 5 ml with glass distilled water.

h) Allow tubes to stand for at least 30-45 minutes in room light before reading.

i) Fluorescence is stable for at least another 30-60 minutes.

j) The tubes should not be read before the stated time lapse or erratic results will be obtained - also the tubes must not be shielded from light.

k) A second set of 7 tubes is set up in the same manner with the following exceptions; 1 ml of 2 M acetate buffer, pH 3.5 is substituted for the pH 6.5 buffer.

l) All tubes are read at the same wavelength. The first set of tubes (pH 6.5) is standardized with tube #1 which is set at 8.0 (or 80) on the microammeter. All other tubes are read against this setting.

m) For the second set of tubes (pH 3.5), tube #3 is set at 8.0 (or 80) on the microammeter. All other tubes are read against this setting.

5. Filter Combinations

a) Primary set - for excitation

1) Corning 3-75

2) Corning 7-51

b) Secondary set - for emission

1) Corning 3-71

2) 498 interference filter

c) Samples are read on a Farrand Model A-2 filter

fluorimeter. Only one cuvette is used and this is rinsed 3 times with glass distilled water after each reading.

6. Calculations

a) Both epinephrine and norepinephrine from fluorescent indoles at pH 6.5. At pH 3.5 most of the NE is decomposed.

b) Calculation of Results

1) Two simultaneous equations are used.

(a/b) NE + (C/d) E = reading of 0.1 ml eluate at pH 6.5.

(e/b) NE + (f/d) E = reading of 0.1 ml eluate at pH 3.5.

a = reading of 0.1 μg NE at pH 6.5 (internal standard)

b = concentration of NE standard = 1 μg/ml.

c = reading of 0.1 μg E at pH 6.5 (Estimated internal standard)

i.e., external standard corrected for same degree of quenching as occurred for NE).

d = concentration of E standard = 1 μg/ml

e = reading of 0.1 μg NE at pH 3.5 (external standard)

f = reading of 0.1 μg E at pH 3.5 (internal standard)

NE = concentration of NE in eluate in μg/ml

E = concentration of E in eluate in μg/ml

c) Sample calculation

1) Theoretical readings

(a) pH 6.5

Tube	Readings	
1	80-5 = 75/2 = 37.5	38.3/0.1 µg NE
2	44-5 = 39	
3	53-5 - 48/2 - 24	23.5/0 µg E
4	28-5 = 23	
5	5	
6	36-8 = 28/2 = 14	10.1 ml eluate
7	8	

(b) pH 3.5

Tube	Readings	
1	8-6 = 2/2 = 1.0	0.8/0.1 µg NE
2	6.5 - 6 = 0.5	
3	80-6 - 74/2 = 37	37/0.1 µg E
4	43-6 = 37	
5	6	
6	16-10 = 6/2 = 3	10.1 ml eluate
7	10	

2) Therefore:

a = 38.3

b = 1 µg/ml

c = 23.5

d = 1 µg/ml

e = 0.8

f = 37

3) Substituting in the equations

$38.3\ NE + 23.5\ E = 14.5$

$0.8\ NE + 37\ E = 3.0$

4) Solution of equations for epinephrine (E) and norepinephrine (NE)

(a) $NE = \frac{14.5 - 23.5\ E}{38.3}$

(b) Substitute (a) in the second equation and solve for E

$0.8\ \frac{(14.5 - 23.5\ E)}{38.3} + 37\ E = 3.0$

$0.3028 - 0.4908E + 37E = 3.0$

$36.5092E = 26972$

$E = 0.0738$ µg/ml of eluate

(x) cc of eluate x $\frac{\text{Total volume}}{\text{Aliquot volume (25 cc)}}$

= µg E/24 hrs.

(c) Solving for norepinephrine

$\frac{14.5 - (21.7 \times 0.0738)}{38.3} = 0.3367$ µg/ml of eluate

(x) cc of eluate x $\frac{\text{Total volume}}{\text{Aliquot volume (25 cc)}}$

= µg NE/24 hrs.

R. Preparation of Solid Samples for Analysis

The 24 hour diets identical to that consumed by the subject (same food lot number and preparation technique) were homogenized. A fractional aliquot was weighed and lyophylized by means of a

Virtis Unitrap freeze-dry system. Dry weight was then determined. An aliquot of the dry specimen equivalent to 1.0 gm of wet specimen was then digested in 50 ml volumetric flask by boiling in 2 ml of concentrated nitric acid for 15 minutes. The digestate was then diluted to volume.

Stool specimens were prepared in a similar manner.

The digested and diluted samples were then analyzed for $Na,^{+}$ $K,^{+}$ $Ca,^{++}$ Mg^{++} by techniques described previously.

Nitrogen analyses are planned on the unaltered wet homogenate when satisfactory autoanalyzer methodology can be developed. Extensive work with this method has not yet resulted in acceptable recovery results.

IV. METABOLIC DIETETIC PROGRAM

A. Dietetic Data

1. Principles of the Balance Diet:

The diet for all study volunteers consisted chiefly of a formula prepared in the research kitchen. Two basic diets were devised, a 2000 calorie diet and a 2500 calorie diet, with the hope that each subject would maintain his weight within a kilogram for the duration of the study. Both diets were calculated according to the following prescription: 80 grams protein, 40% of the total calories as fat, 3% of the total calories as linoleic acid, 1 gram calcium, and a starch-sugar ratio of 1:13. After interviewing the volunteer upon admission, the research dietician decided which of the two calorie levels to use on the basis of the individual's height and weight. Each subject remained on the balance diet for 23 days, the first 2 days of which served as an equilibration period. He was instructed to eat everything on his tray and to rinse containers afterward with distilled water and consume the rinse.

Prior to August 1965, a slightly different diet was used which had approximately 2000 calories, 100 grams protein, 36% of the total calories as fat, 3% of the total calories as linoleic acid, and 1.8 grams calcium. The diet was altered because it was determined that the calcium level was in excess of that planned for the Gemini Program. The basic menu pattern remained the same throughout the entire study; only the formula recipe was revised.

2. Formula Composition and Preparation:

The formula was comprised of the following ingredients: instant powdered skim milk, baby beef, salt-free butter, cottonseed oil, olive oil, canned peaches, Dexin (a high-dextrin powder manufactured by Burroughs-Wellcome & Co.), granulated sugar, distilled water, and flavoring. A dozen or so imitation flavorings were employed to provide some daily variety to the formula.

The ingredients used for the duration of each study were derived from the same lot. Each item in the diet was weighed on a Mettler balance with the exception of the distilled water which was measured in a graduate. The formula ingredients were transferred to a 5-quart Waring Blendor, and the measured distilled water heated and used to rinse each container thoroughly. The homogenized formula was refrigerated for several hours until the foam subsided, then it was weighed into individual glasses. Since it was difficult to remove all of the formula from the blendor, 1 1/4 times the recipe was prepared. It was helpful to have some extra formula readily available for replacement purposes in the event that a patient spilled some. The formula was divided into 4 feedings - 8 AM, 12 Noon, 5 PM and 9 PM.

3. Total Balance Diet:

In addition to the formula, the diet included three Melozet wafers per day, tomato soup at the noon meal, Midget mints, and instant coffee or tea. Melozets (manufactured by the Quinton Co.) are methylcellulose wafers which served a dual purpose in

that they provided something solid in the diet which the patient could chew and at the same time helped somewhat to prevent constipation. The mints were useful in cutting the after-taste left by the formula. Each volunteer had a choice of coffee or tea (or neither beverage, if so desired) with or without Coffee-mate and/or sugar. Otherwise, the diet was standard from patient to patient.

To supplement the balance diet, the patients received 2 Hexavitamin pills daily.

BASIC FORMULA RECIPE FOR BALANCE DIETS USED FROM JANUARY-AUGUST, 1965

Ingredient	Grams
Powdered skim milk (instant)	135.0
Baby beef (Beech-Nut)	345.0
Bexin	52.0
Cottonseed oil	5.0
Olive oil	10.0
Butter, salt-free	55.0
Peaches, sw. cn (heavy syrup)	200.0
Sugar, Granulated	22.0
Distilled water	976.0

The prepared formula was weighed into 8 glasses, 225 grams in each.

NOTE: If the patient took his coffee or tea with Coffee-mate and/or sugar, the formula recipe was adjusted accordingly by decreasing the butter and/or sugar.

CALCULATED COMPOSITION OF A SAMPLE 2000-CALORIE BALANCE DIET USED

FROM JANUARY-AUGUST, 1965

Calculations below based on Coffee 3 times daily

Common Nutrients	Amount
Calories	2008
Nitrogen	15.89 gm.
Protein	99.8 gm.
Fat	80.9 gm.
Carbohydrate	220.1 gm.
Sodium	2793.0 mg or 121.4 mEq
Potassium	3675.0 mg. or 94.2 mEq
Calcium	1817.0 mg. or 90.7 mEq
Magnesium	344.0 mg. or 28.3 mEq
Phosphorus	1879.0 mg.
Iron	9.1 mg.
Vitamin A	3662.0 IU
Vitamin D	22.0 IU
Thiamine	0.59 mg.
Riboflavin	2.85 mg.
Niacin	17.89 mg.
Ascorbic acid	32.0 mg.
Cholesterol	388.0 mg.

Lesser Nutrients	
Vitamin B_6 (pyridoxine)	1.7 mg.
Pantothenate	6.4 mg.
Biotin	33.8 mcg.
Folate	40.2 mcg.
Choline	248.8 mg.
Inositol	231.1 mg.
Vitamin B_{12}	9.5 mcg.
Copper	3.1 mg.
Iodine	1.073 mcg.

NOTE: Some of the above totals are slightly different with tea as a beverage or with neither coffee nor tea.

Hexavitamin Pills: 2 daily provide the following supplemental nutrients.

Vitamin A	10,000 IU	Thiamine	4 mg.
Vitamin D	800 IU	Riboflavin	6 mg.
Ascorbic acid	150 mg.	Niacin	40 mg.

BASIC FORMULA RECIPE FOR BALANCE DIETS USED SINCE AUGUST, 1965

Ingredient	Grams	
	2000 calories	2500 calories
Powdered skim milk (instant)	75.0	75.0
Baby beef (Beech-nut)	340.0	340.0
Dexin	56.0	82.0
Cottonseed oil	5.0	7.0
Olive oil	10.0	12.0
Butter, salt-free	65.0	88.0
Peaches, sw. cn. (heavy syr.)	300.0	400.0
Sugar, granulated	29.0	58.0
Distilled water	920.0	1138.0

Notes:

1. For the 2000 calorie diet, the prepared formula was weighed into 8 glasses, 225 grams in each. For the 2500 calorie diet, the prepared formula was weighed into 8 glasses, 275 grams in each.

2. If the patient took his coffee or tea with Coffee-mate and/or sugar, the formula recipe was adjusted accordingly by decreasing the butter and/or sugar by the necessary amount.

3. The amount of flavoring used varied from one flavor to another. The distilled water was decreased proportionately by the amount of flavoring needed.

CALCULATED COMPOSITION OF BALANCE DIETS USED SINCE AUGUST, 1965

Calculations below based on Coffee 3 times daily

Common Nutrients	Amount	
	2000 calorie	2500 calorie
Calories	1996	2497
Nitrogen	12.63 gm.	12.72 gm.
Protein	78.9 gm.	79.4 gm.
Fat	88.8 gm.	100.9 gm.
Carbohydrate	220.2 gm.	295.2 gm.
Sodium	2643.0 mg. or 114.9 mEq	2647.0 mg. or 115,1 mEq
Potassium	2761.0 mg. or 70.8 mEq	2892.0 mg. or 74.2 mEq
Calcium	1047.0 mg. or 52.2 mEq	1056.0 mg. or 52.7 mEq
Magnesium	265.0 mg. or 21.8 mEq	272.0 mg. or 22.4 mEq
Phosphorus	1290.0 mg.	1306.0 mg.
Iron	9.1 mg.	9.4 mg.
Vitamin A	4404.0 IU	5593.0 IU
Vitamin D	26.0 IU	35.0 IU
Thiamine	0.39 mg.	0.40 mg.
Riboflavin	1.79 mg.	1.81 mg.
Niacin	18.0 mg.	18.6 mg.
Asorbic acid	30.0 mg.	33.0 mg.
Cholesterol	408.0 mg.	465.0 mg.

Lesser Nutrients		
Vitamin B_6 (pyridoxine)	1.5 mg.	1.5 mg.
Pantothenate	4.4 mg.	4.4 mg.
Biotin	24.4 mcg.	24.6 mcg.
Folate	38.8 mcg.	38.8 mcg.
Choline	259.3 mg.	260.7 mg.
Inositol	327.1 mg.	423.1 mg.
Vitamin B_{12}	8.8 mcg.	8.8 mcg.
Copper	2.3 mg.	2.4 mg.
Iodine	0.977 mcg.	1.142 mcg.

NOTE: Some of the above totals are slightly different with tea as a beverage or with neither coffee or tea.

Hexavitamine Pills: 2 daily provide the following suppemental nutrients.

Vitamin A	10,000 IU	Thiamine	4 mg.
Vitamin D	800 IU	Riboflavin	6 mg.
Ascorbic acid	150 mg.	Niacin	40 mg.

SAMPLE MENUS

2000 calorie diet

Breakfast:

450 gm. Formula (2 glasses)
1 Melozet wafer
2.5 gm. instant coffee
200 cc. hot distilled water
10 gm. mints (to be consumed by end of day)

Lunch:

450 gm. Formula (2 glasses)
1 Melozet wafer
200 gm. Tomato soup (100 gm. soup plus 100 cc dist. water)
2.5 gm. instant coffee
200 cc. hot distilled water

Dinner:

450 gm. Formula (2 glasses)
1 Melozet wafer
2.5 gm. instant coffee
200 cc. hot distilled water

Evening Nourishment:

450 gm. Formula (2 glasses)

2500 calorie diet

Breakfast:

550 gm. Formula (2 glasses)
1 Melozet wafer
2.5 gm. instant coffee
200 cc. hot distilled water
10 gm. mints (to be consumed by end of day)

Lunch:

550 gm. Formula (2 glasses)
1 Melozet wafer
200 gm. Tomato soup (100 gm. soup plus 100 cc. dist water)
2.5 gm. instant coffee
200 cc. hot distilled water

Dinner:

550 gm. Formula (2 glasses)
1 Melozet wafer
2.5 gm. instant coffee
200 cc. hot distilled water

Evening Nourishment:

550 gm. Formula (2 glasses)

DIETS OF NASA VOLUNTEERS STUDIED FROM JANUARY, 1965 THROUGH JUNE, 1966

Volunteer	Dates of hospitalization	Dates on Diet	Diet	Notes
A	1/25-2/20 1965	1/25 thru 2/17	2000 cal., 3.1 gm. Ca	This diet is totally different from those which follow.
B	5/23-6/18 "	5/25 thru 6/16	2000 cal., 1.8 gm. Ca	Second cardiac cath. not completed.
C	11/14-1/2 1966	11/16 thru 12/14	2500 cal., 1.0 gm. Ca	Extra 6 days on diet, revised version.
1.	2/21-3/27 1965	2/23 thru 3/17	2000 cal., 1.8 gm. Ca	Error on 3/10-extra 4.5 gm. sugar given.
2.	3/21-4/18 "	3/23 thru breakfast 4/14	2000 cal., 1.8 gm. Ca	Cardiac cath. done in the afternoon.
3.	4/4-5/1 "	4/6 thru 4/27	2000 cal., 1.8 gm. Ca	
4.	5/9-6/6 "	5/11 thru 6/2	2000 cal., 1.8 gm. Ca	Error on 5/22-rec'd 5 gm. butter which he ate.
5.	6/7-7/3 "	6/9 thru 6/30	2000 cal., 1.8 gm. Ca	
6.	6/19-7/17 "	6/22 thru 7/14	2000 cal., 1.8 gm. Ca	Error on 7/8-patient failed to eat 4 gm mints.
7.	8/14-9/11 "	8/17 thru 9/8	2000 cal., 1.8 gm. Ca	
*8.	8/29-10/1 "	8/31 thru 9/22	2000 cal., 1.0 gm. Ca	Revised diet.
9.	9/11-10/9 "	9/14 thru 10/5	2500 cal., 1.0 gm. Ca	
10.	10/17-11/20 "	10/19 thru 11/14	2000 cal., 1.0 gm. Ca	Spent extra 4 days on bedrest and diet.
11.	10/31-12/4 "	11/2 thru 11/30	2000 cal., 1.0 gm. Ca	Extra 6 days on diet.
12.	1/2-2/5 1966	1/4 thru 1/26	2500 cal., 1.0 gm. Ca	

* First patient to receive revised diet.

DIETS OF NASA VOLUNTEERS (continued)

Volunteer	Dates of hospitalization	Dates on Diet	Diet	Notes
13.	1/16-2/9 1966	1/17 thru 2/12	2000 cal., 1.0 gm. Ca	
14.	1/30-3/5 "	2/1 thru 2/23	2500 cal., 1.0 gm. Ca	Error on 2/5 - Patient failed to eat 10 gm. mints.
15.	2/20-3/19 "	2/22 thru 3/16	2000 cal., 1.0 gm. Ca	
16.	3/27-5/1 "	3/29 thru 4/21	2500 cal., 1.0 gm. Ca	
17.	4/17-5/21 "	4/19 thru 5/11	2000 cal., 1.0 gm. Ca	
18.	5/2-6/4 "	5/3 thru 5/25	2000 cal., 1.0 gm. Ca	
19.	5/15-6/19 "	5/17 thru 6/8	2500 cal., 1.0 gm. Ca	
20.	6/5-7/9 "	6/7 thru 6/29	2500 cal., 1.0 gm. Ca	

CALCULATED TOTALS OF COMMON NUTRIENTS IN BALANCE DIETS OF VOLUNTEERS

		Grams			Milligrams				
Volunteer	Cal.	PRO	FAT	CHO	Na	K	Ca	P	Mg
A	2006	100.4	70.0	243.6	2390	4694	3135	2560	365
B	2008	99.8	80.9	220.1	2793	3675	1817	1879	344
C	2497	79.4	110.9	295.2	2647	2892	1056	1306	272
1.	2007	100.4	80.6	220.1	2793	3684	1818	1885	344
2.	2013	100.4	81.2	220.1	2793	3684	1818	1885	344
3.	2013	100.4	81.2	220.1	2793	3684	1818	1885	344
4.	2013	100.4	81.2	220.1	2793	3653	1813	1876	337
5.	2008	99.8	80.9	220.1	2793	3675	1817	1879	344
6.	2008	99.8	80.9	220.1	2793	3675	1817	1879	344
7.	2008	99.8	80.9	220.1	2793	3675	1817	1879	344
8.	1996	78.9	88.8	220.2	2643	2761	1047	1290	265
9.	2497	79.4	110.9	295.2	2644	2729	1046	1287	261
10.	1996	78.9	88.8	220.2	2638	2517	1034	1261	231
11.	1996	78.9	88.8	220.4	2638	2667	1034	1261	238
12.	2497	79.4	110.9	295.2	2647	2892	1056	1306	272
13.	1996	78.9	88.8	220.4	2638	2667	1034	1261	238
14.	2497	79.4	110.9	295.2	2642	2648	1043	1277	238
15.	1994	78.9	88.6	220.2	2617	2666	1033	1260	238
16.	2497	79.4	110.9	295.2	2647	2892	1056	1306	272
17.	1996	78.9	88.8	220.2	2643	2761	1047	1290	265
18.	1996	78.9	88.8	220.2	2643	2761	1047	1290	265
19.	2497	79.4	110.9	295.2	2647	2892	1056	1306	272
20.	2497	79.4	110.9	295.2	2647	2892	1056	1306	272

4. Discussion:

The formula-type diet, though not the most palatable, was successful under the circumstances. Every patient accepted the diet for what it was and complaints were minimal. There were no incidents of vomiting and surprisingly few errors in serving the diets. Any errors that were made are recorded on the preceding tables.

B. Other Intake

The volunteer received no oral intake except the prescribed formula diet and distilled water. Smoking was permitted. The dentrifice used was calcium free.

V. DATA ANALYSIS

In all comparisons, a standard two-tailed student (t-test) was utilized. Standard error is always expressed for 95% Confidence Limits.

A. Hemodynamic Data

All recordings were analyzed by manual techniques.

1. Pressure Recordings

Prior to each catheterization procedure, P23D transducers were calibrated by means of a mercury manometer. A series of pressure recordings were made at gain and amplitude settings required to give full scale deflection of the oscilloscopic tracing at desired peak pressures. Pressures recorded were 5,10,15,20,30,50,100 and 200 mm Hg at the range setting 0-200 mm Hg utilized for brachial artery pressures and 5,10,15, 20,30,50 and 100 mm Hg at the range setting of 0-100 mm Hg utilized for pulmonary artery pressures. The deflections obtained were used in subsequent pressure measurements.

As already noted (II-B-7) pressures were recorded at a paper speed of 5 mm/sec. for the most part, with increases to 25 mm/sec. each minute. In the analysis of tracings, the entire one minute interval was examined and the average systolic and diastolic pressure during two consecutive and representative respiratory cycles was determined. This procedure was used in all phases of the catheterization study. The only exception was in determination of pressure when the termination of tilting was

necessitated by hypotension. In this case, the average pressure during the final one or two respiratory cycles prior to tilt-down was determined.

Mean pressures were determined by electronic damping of the pressure pulse immediately after each 25 mm/sec run. The average mean pressures were determined during two representative respiratory cycles.

In addition to the above analysis, all pressure curves obtained at rest and during the 70° passive tilt were analyzed for frequency and amplitude of respiratory and vasomotor waveforms.

2. Heart Rate Recordings

These were obtained as noted above (II-B-7) by means of an EKG coupled R-R interval tachometer. After establishing the baseline (zero) reference, maximum deflection for any given heart rate range setting was obtained by an internal calibration signal. The amplitude of this deflection was adjusted to exactly equal the 200 mm Hg deflection of the arterial pressure amplifier. This allowed the use of the arterial pressure calibration curve for heart rate measurement. The 0-200 range setting was used for heart rate recording in all cases.

Average heart rate during each one minute interval was obtained by the same method as noted above for blood pressure. Heart rate at termination of tilt was also determined in a manner identical to that for blood pressure. Heart rate curves were also analyzed for respiratory and vasomotor waveforms.

3. Analysis of Cardiac Output Curves

The deflection produced by 1.0 mgm indocyanine-green dye/liter of whole blood was determined by plotting the deflection produced by circulating three different concentrations of the dye in whole blood through the cuvette densitometer at withdrawal rates used in vivo. This deflection, mm/1.0 mgm/liter was utilized as the dye calibration factor. The dye curves were then analyzed as follows:

a) The appearance time (AT) was the interval between the instant of injection (T_o) to the time of first detection of dye at the sampling site (T_a).

b) Using the densitometer trace preceding the curve as a baseline, the height of the curve was analyzed at one-second intervals measured from T_a.

c) Each one-second measurement of height was converted to mgm of indocyanine green dye/liter by the following relationship:

$$\text{Concentration Indocyanine Green Dye (mgm/L)} = \frac{\text{Deflection (mm)}}{\text{Dye Calibration Factor}}$$

d) The series of one-second dye concentrations thus obtained were plotted on 3 cycle semi-logarithmic paper. The method of Kinsman, Moore, and Hamilton was used to determine the onset of recirculation (108). The smooth downslope of the dye concentration

curve prior to recirculation was then extrapolated through one cycle to a concentration of 0.1 mgm/L. The contribution of the curve to total concentration is insignificant below this level.

e) The area below the curve in mgm/L was determined by a summation of one-second concentrations from T_a to the time of disappearance, corresponding to 0.1 mgm/L, T_d. This sum is equivalent to $\bar{C}T$, where $\bar{C}$ is average concentration and T is time required to inscribe the curve.

f) CARDIAC OUTPUT was determined by the relationship:

$$\text{C.O. (L/min)} = \frac{60\ I}{\bar{C}T} \quad \text{or} \quad \frac{60\ I}{\text{Curve area (mgm/L)}}$$

where I is the injected quantity of dye in milligrams and 60 (seconds/min) is used to convert that portion of the minute output described during the time (seconds) required to inscribe the curve, T, into cardiac output per minute.

g) Cardiac Index represents cardiac output per M^2 of body surface area. The patient's B.S.A. on the day of the procedure was utilized for this conversion.

h) STROKE VOLUME was determined by dividing the average heart rate during inscription of the cardiac output curve into the cardiac output (ml/min). Since the heart rates noted in the hemodynamic results are

minute averages they are not necessarily the heart rates used to calculate stroke volume.

i) MEAN CIRCULATION TIME was determined from the cardiac output curve as follows:

$$M.C.T. = \frac{(AT + 1)\ (C_1) + (AT + 2)\ (C_2) + (AT + n)\ (C_n)}{\text{Curve Area (mgm/L)}}$$

Each relationship (AT + n) representing the time interval from injection to the individual one-second measurements of curve height (after correction for densitometer dead space time) and (C_n) representing the actual concentration at that time.

j) CENTRAL BLOOD VOLUME is a relative measurement of change within a given individual. The measurement as performed in these studies represents all blood in all vessels between the point of injection and the point of sampling plus the blood in those arteries having the same circulation time from the aortic valve as the sampling site. The majority of the volume measured by the present technique would represent pulmonary and left heart volume. The addition of blood contained in vessels beyond the aortic valve would not alter the significance of changes in "Central Blood Volume" provided the injection and sampling sites are constant. The calculation of "Central Blood Volume" was as follows:

$$\text{C.B.V. (ml)} = \text{Cardiac Output (ml/sec)} \times \text{Mean Circulation Time}$$

$$\text{or C.B.V.} = \frac{\text{CO (ml/min)}}{60} \times \text{M.C.T.}$$

4. Determination of Diastolic Filling Period

   a) Diastolic filling period in seconds per beat was determined as the average interval during two respiratory cycles between the dicrotic notch of one arterial pressure pulse and the onset of the following arterial pressure pulse.

   b) D.F.P./beat was converted to D.F.P. (seconds/minute) by multiplication by the corresponding heart rate.

5. Determination of Vascular Resistance

Vascular resistance may be described as the impedance to blood flow through a given portion of the circulation. In the present studies, available data allows calculation of resistance in both the peripheral and pulmonary vascular systems. Aperia's formula which gives results in absolute units is as follows:

$$\text{Resistance (dynes-sec-cm}^{-5}) = \frac{\text{Pressure Differences (dynes/cm}^2)}{\text{Flow (ml/sec)}}$$

$$\text{or} \qquad R = \frac{(P_1 - P_2) \times 1332}{\dot{Q}}$$

where R = resistance in dynes-sec-cm^{-5}, $P_1 - P_2$ = the pressure loss across the resistance circuit in mm Hg, 1332 the factor for converting to dynes, and $\dot{Q}$ = blood flow in milliliters per second through the circuit (129).

In the present study, the mean systemic arterial pressure was substituted for $(P_1 - P_2)$ in the determination of peripheral vascular resistance. As noted above (V-A-1) mean pressure was determined by electronic damping of the pressure pulse tracing. No allowance was made for any residual pressure at the outflow of the circuit as this was not measured and was undoubtedly small in comparison to the mean arterial pressure.

In the calculation of pulmonary vascular resistance, mean pulmonary arterial pressure was substituted for P_1 and 5 mm Hg was empirically chosen as the mean diastolic pressure in the left ventricle. This was substituted for P_2.

6. Determination of Oxygen Consumption

Samples of expired air obtained during circumstances of rest and exercise were analyzed for oxygen partial pressure (mm Hg) by means of a PO_2 electrode system. The electrode was zeroed with an oxygen free gas mixture.

Partial pressure of oxygen in room air was calculated by the formula: $P_{IO_2} = F_{IO_2} \times (P_B - P_{H_2O})$ where P_{IO_2} is the partial pressure and F_{IO_2} the fractional concentration of oxygen in room air, P_B the barometric pressure and P_{H_2O} the partial pressure of water.

After filling the electrode cuvette with room air, the meter was calibrated to read the calculated partial pressure of oxygen in room air. After satisfactory calibration, the expired

air sample was flushed into the cuvette and PO_2 determined. The fractional concentration of oxygen in expired air ($F_{E_{O_2}}$) was then calculated as follows:

$$F_{E_{O_2}} = \frac{P_{E_{O_2}}}{(P_B - P_{H_2O})} .$$

Minute ventilation ($\dot{V}$) was determined by the relation:

$$\dot{V} = \frac{(V_2 - V_1)}{t} \times f_T \times f_{BTPS}$$

where V_1 is the initial tissot reading, f_T the tissot factor and f_{BTPS} is the factor for correcting the volume to body temperature and pressure saturated with water vapor.

Oxygen consumption was then determined by the equation:

$$\text{Oxygen Consumption (ml/min)} = \dot{V} \times (F_{I_{O_2}} - F_{E_{O_2}}).$$

7. Compilation of 70° Passive Tilt Data

a) Recordings obtained during the tilt procedure were analyzed on a minute to minute basis for:

1) Pulmonary and brachial artery pressure
2) Heart rate
3) Respiratory rate
4) Diastolic filling period
5) Amplitude and frequency of vasomotor waveforms

b) Based on calculations made from cardiac output studies, heart rate and mean pressure, alterations in the following parameters were evaluated at 5, 10 and 18 minutes:

1) Cardiac output and cardiac index
2) Mean circulation time
3) Central blood volume
4) Stroke volume
5) Pulmonary and peripheral vascular resistance

In all comparisons, the supine resting data acquired prior to tilting served as the basal reference.

8. Compilation of Exercise Data

a) Parameters which were analyzed included:

1) Heart rate, blood pressure at various exercise levels in all subjects.
2) Oxygen consumption during 30 or 50 watt steady state exercise in all subjects.
3) Oxygen consumption during 75 and 100 watt steady state exercise in subjects 12 to 20.
4) Cardiac output during 30 or 50 watt steady state exercise in all subjects.

In the presentation of alterations in heart rate and blood pressure, the resting measurement immediately before onset of exercise was used as the basal reference

point. In the case of cardiac output and oxygen consumption, the supine resting values obtained prior to the tilt procedure served as basal reference points.

b) In addition to evaluating the above data in terms of alterations from the basal state induced by exercise in the pre- and post-recumbency state, the following derived data was evaluated:

1) Pulmonary and peripheral vascular resistance

2) The VENTILATORY EQUIVALENT or the respiratory volume per 100 ml of oxygen consumed was determined as follows:

$$\text{V.E. (L/100 ml } O_2) = \frac{\text{Ventilation (L/min)}}{O_2 \text{ Consumption (ml/min)}} \times 100$$

3) The EXERCISE FACTOR or the increase in cardiac output per 100 ml increase in oxygen consumption was determined by the relation:

$$\text{EXERCISE FACTOR } (\Delta \text{ C.O./100 ml } O_2) = \frac{\text{C.O.}_{Ex.} - \text{C.O.}_{Rest} \text{ (L/min)}}{O_2 \text{ Cons(Ex)} - O_2 \text{ Cons(Rest)}} \times 100 .$$

4) LEFT VENTRICULAR WORK was determined from the brachial artery mean pressure and cardiac output by the formula:

$$\text{L.V.W. (KgM/min)} = \frac{\text{B.A.}_m \text{ (mm Hg)} \times \text{C.O. (ml/min)} \times 13.6}{100{,}000}$$

9. Compilation of Data From Tyramine Stimulation Test

Recordings were analyzed on a minute to minute basis for:

a) Pulmonary arterial pressure

b) Brachial arterial pressure

c) Heart rate

Baseline data immediately before injection was used for comparison.

Results from urine and plasma catecholamine analysis are not presented. In the case of the former, subjects were only rarely able to void prior to tyramine injection. Thus, subsequent urine collection was contaminated by tilt and exercise urine. Plasma results were not considered valid as noted above (III-P).

10. Analysis of Valsalva Results

All tracings were analyzed for response in brachial and pulmonary artery pressure response to controlled Valsalva maneuver. Heart rate response was similarly monitored. Represenative results are presented.

B. Analysis of Balance Data

1. Data is presented for 24 hour balance for the following parameters:

a) Water

b) Sodium

c) Potassium

d) Calcium

e) Magnesium

f) Chloride

g) Phosphate

2. Nitrogen balance data is not presented due to the aforementioned difficulties with planned methodology. These will be submitted as an addendum report when methodologic problems are resolved.

3. Balance data was analyzed in a sequential manner as follows:

a) Individual Data

1) Daily balance for each individual was calculated by the formula:

Balance = Total 24-Hour Intake - Total 24-Hour Output.

Intake was usually represented only by dietary intake except on clearance days when additional sodium and chloride intake was present.

2) Cumulative balance was determined separately for the Ambulant and Bedrest Phases. In the case of sodium, this data was determined without correction and by correction for 20 meq/day of extrarenal sodium loss. This figure was arrived at from results of calculated mean daily sodium balance of all subjects during the Ambulant Phase. The predominant loss would be via sweating. No correction was made in the case of other electrolyte data.

b) Group Data

Results for all subjects in the control and 9-α-fluorohydrocortisone groups were determined for the following parameters:

1) Average 24-Hour Urinary Excretion

This was determined separately for the Ambulant and Bedrest Phases of each subject. The mean result of each group was then derived from individual averages. This analysis was performed on all electrolyte and water balance data.

2) Average 24-Hour Balance

This was determined separately for the Ambulant and Bedrest Phases of each subject. The mean result for each group was then derived from the individual averages. This analysis was performed for water, sodium, potassium, calcium and magnesium. It was not determined for chloride and phosphate because of the lack of analytic data for diet and feces.

3) Mean Daily Sodium and Water Balance

This was determined on each day of each phase from daily group averages.

C. Clearance Data

1. P.A.H. and Inulin

This study was discontinued after subject 9 because of erratic results due to incomplete bladder emptying. This was particularly noticeable during the Bedrest Phase. Results on the initial nine subjects are considered invalid for this reason. Therefore, they have been discarded.

2. 24-Hour Clearance

These were determined on Ambulant Day 1 and Bedrest Day 1 and 12 for the following parameters:

a) Endogenous Creatinine

b) Phosphate

c) Osmotic

d) Free Water

Individual values for each subject as well as group averages are presented.

D. Catecholamine Data

1. Individual Results - Urinary Catecholamines

Values for norepinephrine and epinephrine are presented for each day of study in the individual subject.

2. Group Results - Urinary Catecholamines

For purposes of group analysis, each subjects values during a given week of study were averaged. The group mean for each week was then derived from the individual average result. This type of analysis was performed for epinephrine and norepinephrine.

E. Blood Volume Determination

1. The dose syringe was counted for 5 minutes before and after injection of the dose into the subject.

2. Three milliliter aliquots of blood samples obtained from the patient before dose injection and at 10,20 and 30 minutes after injection were similarly counted.

3. Hematocrits were obtained from each sample and corrected for trapped plasma.

4. Plasma Volume Calculation:

a) $\text{Dose(CPM)} = \frac{\text{CPM Full Syringe - CPM Empty Syringe}}{5 \text{ min.}}$

b) $\text{Sample (CPM)} = \frac{\text{CPM Sample - CPM Blood Background}}{5 \text{ min} \times 3 \text{ ml}}$

c) The 10,20 and 30 minute sample counts were then plotted on linear graph paper. A line of best fit was drawn through the three points to time zero. The counts per minute at T_o were then utilized in calculating total blood volume by the formula:

$$\text{Total Blood Volume (ml)} = \frac{\text{Dose (CPM)}}{\text{Sample } T_o \text{ (CPM)}} .$$

d) Plasma volume was then determined by:

Plasma Volume (ml) = Uncorrected Blood Volume x 1.00 - Hematocrit.

e) Total blood volume was corrected to correspond more nearly with total body rather than peripheral hematocrit by the equation:

Corrected Blood Volume = Total Blood Volume x 0.95.

f) Red blood cell volume was then calculated by the relation:

R.B.C. Volume (ml) = Corrected Total Blood Volume - Plasma Volume.

g) In the analysis of total blood volume, plasma volume and red cell volume results, individual values for any given day of study were used to determine group means for that day. Composite means for each group were derived for all values obtained during a given interval of the study. The values used for these intervals were as follows:

1) Ambulant - Values from Ambulant Day 1 and 7 and Bedrest Day 1.

2) Bedrest Week 1 - Values from Bedrest Day 2 and 6.

3) Bedrest Week 2 - Values from Bedrest Day 11 and 15.

F. Additional Data

The following data has been compiled and evaluated by examination only. No attempt was made to evaluate it statistically for the purpose of this report. Its value and pertinence will be discussed under Results.

1. 17-Hydroxycorticosteroid Excretion

2. Aldosterone Excretion

3. 24-Hour Urine Creatinine

4. 24-Hour Urine Osmolality

5. Blood Chemistry

   a) Na^{+}

   b) K^{+}

   c) Cl^{-}

   d) PO_4^{--}

   e) Ca^{++}

   f) Mg^{++}

   g) Osmolality

   h) Creatinine

   i) Serum Proteins

   j) Cholesterol

   k) Triglycerides

6. Hematologic Data

VI. RESULTS

A. General

Table 1 shows basic data on each subject as regards height, average blood pressure during ambulation and bedrest as well as the duration of bedrest. Treatment or non-treatment with 9-α-fluorohydrocortisone (9-α) is also indicated. It is apparent that average systolic and diastolic blood pressure showed no trend resultant either from bedrest or treatment with 9-α. Subjects 10 and 13 were maintained at bedrest beyond the usual 14 days because of acute febrile illness at the time the study would normally have been terminated. Metabolic data evaluation is compiled only for the planned duration of study.

Table 2 shows the weight of each subject on Ambulation Day 1 and Bedrest Days 1 and 15. Mean change in weight during the Ambulation and Bedrest Phases is presented for each group. Weight loss in the control group was approximately twice as great during bedrest as during ambulation. The 9-α group showed a mean increase in weight during bedrest while showing a weight loss similar to the control group during ambulation.

Table 3 reviews the general problems which occurred during the study. Bedrest compliance was excellent. It was necessary to terminate only one study (Subject Y) because of non-compliance. Backache and constipation were frequent complaints as was headache. Problems with flatulence were primarily noted at the beginning of bedrest and were probably related to excessive use of air swallowing to propel food. Several patients experienced minor difficulty

in voiding for morning urine close-out during bedrest. This problem was serious in only one patient (Subject B). Catheterization was required in this subject on one occasion. One subject (Subject C) developed typical renal colic eight days after completion of bedrest. He subsequently passed a stone which was determined to be calcium-oxylate. There was no prior history of renal disease. However, the same subject (Subject C) was found to have chronic hepatitis during the course of the study. This was manifested by persistently elevated S.G.O.T. and thymol turbidity. The diagnosis was confirmed by liver biopsy. One additional subject (Subject 10) developed acute cystitis with hematuria on the eleventh day of bedrest. Retrospectively, the patient recalled a similar episode 4-6 months previously. Two febrile episodes were associated with acute upper respiratory infection (Subjects 12 and 13).

Problems associated with cardiac catheterization were minimal although a number of subjects had mild phebitis in the vein utilized for catheterization. This problem responded readily to warm moist compresses. One episode of incomplete atrio-ventricular block occurred during a pre-bedrest catheterization (Subject X). One episode of catheter induced atrial fibrillation occurred during post-recumbency catheterization (Subject B). The procedure was cancelled and atrial fibrillation persisted until 0.5 mgm of intravenous digoxin was given four hours later. There was a general impression of increased myocardial irritability to catheter

passage during post-recumbency catheterization. This is difficult to confirm. However, pulmonary artery catheterization was accomplished in all subjects pre-recumbency, while irritability made it necessary to leave the catheter in the right atrium in four post-recumbency studies (Subjects 11,13,15,17). The interpretation of this outcome is difficult due to variation in operator technique.

B. Results of Hemodynamic Studies

1. 70° Passive Tilt

Table 4 shows the tilt tolerance of each subject in the control and 9-α treated groups before and after bedrest. The average tolerance in minutes of each group for each study is shown. The notable feature of this table is the 50% incidence of tilt intolerance in each group before as well as after bedrest. The average duration of tilt tolerance is likewise almost identical. Close perusal of this table reveals that only two of the initial six control subjects tolerated the post-recumbency tilt whereas five out of the initial six treated subjects were able to tolerate the planned duration of the tilt. At this point 9-α appeared to be exerting a beneficial effect. The failure of the final four treated subjects to tolerate post-recumbency tilting accompanied by the tilt tolerance of three of the last four control subjects raised a question as to the possibility of reversal of placebo and drug in the final eight subjects. This possibility was rapidly excluded by pharmacy and nursing records. The further possibility

of deterioration of 9-alpha-fluorohydrocortisone was investigated. The manufacturer* specifies a potency of 90-110% of labeled potency. Analyses performed on tablets returned to the manufacturer revealed an average potency of 89% when evaluated by two separate techniques. Therefore, the data on tilt tolerance may be accepted as valid.

Table 5 contains the absolute results for: brachial and pulmonary arterial pressure; heart rate; cardiac output; stroke volume; mean central blood volume; diastolic filling period; and peripheral and pulmonary resistances for control group subjects at specified time intervals during the course of tilting.

Table 6 shows similar data for the 9-alpha-fluorohydrocortisone group.

Table 7 and 8 contain pulse pressure data at one, two, five, ten, fifteen, eighteen and the final minute of tilt.

The typical initial response to tilting of brachial arterial pressure in both groups was a rise in both systolic and diastolic pressure, with a slightly greater rise in the latter in most cases. This resulted in diminished pulse pressure. Subsequent changes generally consisted of some degree of fall in systolic, diastolic and pulse pressure. Neither the control or treated subjects nor the pre- or post-bedrest studies can be differentiated on the basis of arterial pressure response to tilting. The occurrence of syncope cannot be predicted on the basis of differences in the sequential alterations in systolic,

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*E.R. Squibb

diastolic or pulse pressure shown in Tables 5-8. However, it should be noted that the majority of subjects with tilt intolerance had a fall in pulse pressure to lower levels than the tolerant subjects. This decrease in pulse pressures usually occurred several minutes before syncope (or pre-syncope).

No characteristic alteration in pulmonary artery pressure was noted in relation to tilting. In four cases, all post-recumbency (Subjects 11,13,15,17), the catheter was left in the right atrium due to ventricular irritability during attempted pulmonary artery catheterization. In two of these cases (Subjects 11,17), both post-recumbency and both showing tilt intolerance with pre-syncope, there was a definite fall in right atrial mean pressure early in the tilt. In the remaining two cases (Subjects 13,15), right atrial mean pressure was relatively stable throughout the tilt procedure. Both subjects tolerated the tilt procedures for twenty minutes.

Heart rate, the most easily measured parameter, showed the most obvious and consistent changes in relation to tilting. In all pre-recumbency studies, there was an immediate rise in heart rate above basal levels. This tended to be slightly progressive throughout the tilt. Five of the ten pre-recumbency hypotensive episodes were associated with a drop in heart rate (vasodepressor syncope), the remaining five were associated with a further rise or no fall in heart rate (arterial anemic hypotension).

Following bedrest, the rise in heart rate associated with tilting was much greater than before bedrest. Seven of the ten post-recumbency hypotensive episodes were of the vasodepressor syncope type, the remainder were unassociated with a fall in heart rate. Since both types of response were seen in some subjects it is possible that the true vasodepressor response would have occurred in all cases had the tilt been maintained.

Diastolic filling period tended to parallel heart rate reciprocally in most studies, with a greater time per minute devoted to systole as heart rate increased during the tilt. Thus, the time per minute allowed for diastolic filling of the ventricles was reduced. There was no correlation between the degree of shortening of D.F.P. and the occurrence of hypotensive episodes.

In general, tilting was accompanied by a fall in cardiac output, stroke volume and central blood volume as well as a rise in peripheral and pulmonary vascular resistance and heart rate. The results of measurement of these parameters were available on most subjects after 5 minutes of tilt. This time interval was, therefore, chosen to determine the average data for the control and 9-α groups. Table 9 shows a comparison of the two groups in terms of alterations produced by five minutes of tilting before and after bedrest. In determining group averages, a subject's data was used only if both pre- and post-recumbency results for the parameter were available. Thus, data from subjects 4 and 12

of the control group and subjects 16, 18, 20 of the treated group were excluded from the calculation of group averages for cardiac output, central blood volume, stroke volume, and peripheral vascular resistance. Subject 4 of the control group and 20 of the treated group were excluded from heart rate calculations. Subjects 4,12,13,15 of the control group and 11,16,17,18,20 of the treated group were excluded from calculation of average pulmonary vascular resistance.

The two groups were analyzed to determine if they were comparable on the basis of resting values before as well as after bedrest. In no case was there a statistically significant difference between the supine rest values. The change in each parameter induced by tilting was also analyzed to determine if 9-α affected this change. This evaluation showed no statistically significant effect on any parameter studied. Thus, the two groups actually constituted a single homogeneous group.

The composite hemodynamic data for all subjects is shown in Table 10. This table presents the mean results of all subjects having both pre- and post-recumbency values for each interval studied (5,10,18 minutes). It should be recalled that cardiac output determinations were not made at 10 minutes of tilt until Subject 9. This explains the smaller number of values at 10 minutes than at 18 minutes. Graphic data presentation is seen in Figure 2.

No parameter showed a significant alteration in supine resting values by bedrest or prior exposure to the study. The

absolute level reached in response to tilt was altered by bedrest only in the case of heart rate and stroke volume, the former showing a significantly higher level at 5,10,18 minutes and the latter a significantly lower level at 5 and possibly at 10 minutes ($P < 0.1 > 0.05$). At 18 minutes, the stroke volume was not significantly lower when compared with pre-recumbency studies. It should be recalled that the 18 minute values are from the more stable subjects.

In terms of change from supine resting values, the fall in cardiac index during tilt was statistically insignificant prior to bedrest at any interval studied. Post-recumbency tilting resulted in a fall in cardiac index which was statistically significant at 5 and 10 minutes of tilt. The change was not significant at 18 minutes, again possibly due to the fact that the values came from the more stable subjects.

The decrease in central blood volume was significant before and after bedrest at 5 and 10 minutes, with evidence of a larger continuing fall at 10 minutes of the post-recumbency tilt. At 18 minutes the values, as in the case of cardiac output, no longer showed a significant decrement from supine values. The small sample studied may tend to mask significant changes even in this more stable group of subjects.

Highly significant decreases in stroke volume and increases in heart rate as a result of tilting were noted at all time intervals pre- and post-recumbency. The magnitude of change was significantly

greater post-recumbency.

Peripheral vascular resistance showed a significant increase at 5 minutes of tilt before as well as after bedrest, the incremental change again being larger after bedrest. At 10 and 18 minutes the changes were not statistically significant even though numerically large. This is undoubtedly related to the wide spread of resistance values in the small number of subjects used for analysis.

Pulmonary vascular resistance showed a significant increase after 5 minutes of tilting during the post-recumbency study. No significant change was noted before bedrest or during the later intervals post-recumbency. Interpretation of the changes at 10 and 18 minutes is complicated by the small number of paired observations.

The magnitude of decrease in cardiac output during tilt is of interest and cannot be determined from the values for cardiac index. Table 11-12 shows that the mean decrease in cardiac output post-recumbency exceeded pre-recumbency decrements by 300 to 700 ml in each group at the time intervals studied. Examination of Table 13 suggests that this post-recumbency decrement cannot be entirely explained by a decrease in central blood volume. Central blood volume showed no decrement from pre-recumbency values at 5 minutes of tilt and only a 200 ml decrement at 10 minutes of post-recumbency tilting, while the decrements in cardiac output at these intervals were 300 ml and 700 ml, respectively.

An interesting phenomena was noted in virtually all subjects during 70° tilts. This consisted of oscillations in blood pressure and heart rate which occurred at a rate of 0-6 times per minute, clearly slower than respiration. Figures 3a & 3b illustrate these "vasomotor waves." It can be seen that they are present only in the brachial artery pressure tracings and are not reflected in the pulmonary artery pressure. Changes in heart rate are reciprocal rising as pressure falls and vice versa. Independent respiratory variations are apparent. In an attempt to evaluate the relationship of these waveforms to tilt intolerance, subjects were divided into stable and hypotensive groups. Those with syncope or pre-syncope during pre-recumbency tilting made up the pre-recumbency hypotensive group, while those with syncope during post-recumbency tilt made up the post-recumbency hypotensive group. Table 14 shows the percentage of subjects with vasomotor waves. In the supine position, the characteristic waveform was present in a larger percentage of the hypotensive subjects both before and after bedrest. In both groups, the percentage was higher post-recumbency. During tilt they were present in the large majority of both groups without respect to bedrest status. In the hypotensive group they tended to disappear shortly before syncope. Table 15 shows the frequency of the wave activity in the two groups. In the stable group the waves occurred at a mean rate of 1-2/minute in the supine position, rising to a mean rate of 3-4/minute during tilt. They remained at this frequency

throughout tilt. In the hypotensive group, supine frequency was 0-1/minute, rising to 2-4/minute during the early minutes of tilt and then showing a statistically significant fall to supine frequency during the terminal 2 minutes before the hypotensive episode. There was no statistically significant difference on comparison of pre- and post-recumbency results within the two groups. Table 16 shows the amplitude of brachial artery pressure variation due to the waveforms. Results are expressed as differences between trough and peak of systolic pressure. Supine variations were not significantly effected by bedrest. The absence of waveforms in the pre-recumbency hypotensive group was significantly different from the stable group. On assuming the tilt before bedrest, the hypotensive subjects developed larger magnitude waveforms than did the stable group. The subjects in the stable group showed significantly larger waveforms after bedrest. These were similar in height to those seen in the hypotensive group during both tilts. The striking and important difference between the stable and hypotensive groups lay in the decrease in height of waveforms in the hypotensive group within the final two minutes before the hypotensive episode. This was highly significantly different from results obtained in the stable group. This difference was present both before and after bedrest. The changes in frequency and amplitude of brachial artery "vasomotor waves" are graphically illustrated in Figure 4. As already noted, similar changes were seen in heart rate. These changes are

depicted in Table 17. It is readily apparent that hypotensive subjects had larger magnitude variations than did stable subjects irrespective of bedrest stages. Late in the post-recumbency tilt, the magnitude was less in the stable group than had been the case pre-recumbency. This is felt to be due to masking by the high mean heart rate. Post-recumbency stable subjects and pre- as well as post-recumbency hypotensive subjects show low magnitude variations during the final two minutes of tilt. This is also thought to be related to the tachycardia present in all of these subjects prior to the vasodepressor episode.

2. Exercise Studies

Tables 18 and 19 contain data on individual responses to 50 watt exercise by subjects in the control and 9-α groups, respectively. Parameters considered include cardiac index, central blood volume, stroke volume, heart rate, peripheral and pulmonary vascular resistance. Summary data on group response are presented in Table 20. With the exception of the lower post-recumbency mean resting heart rate in the 9-α group, the two groups do not differ from a statistical viewpoint. Thus, data could be pooled. The results of pooled data are depicted in Table 21. With the exception of failure of stroke volume to rise and pulmonary vascular resistance to fall significantly during post-recumbency exercise, all changes resulting from exercise are highly significant, as expected. The notable alterations following bedrest are the significantly smaller increments in cardiac index and stroke volume due to exercise. Also,

the heart rate shows a significantly greater rise as a result of post-recumbency exercise. Figure 5 summarizes these results graphically.

Left ventricular work during 50 watt exercise was significantly increased in both groups. This data, as well as combined data is presented in Table 22. Left ventricular work response to exercise was not significantly altered by bedrest.

Table 23 depicts the alteration induced in oxygen consumption by 50 watt exercise. Response to exercise was not effected by 9-α or bedrest.

Table 24 shows additional oxygen consumption data at 75 and 100 watt exercise. Results are again remarkably similar in both groups before and after bedrest.

Alterations in ventilatory equivalent due to 50 watt exercise are presented in Table 25. Ventilatory equivalent response was not altered by 9-α. When the combined data is observed it can be seen that a slightly greater amount of ventilation is required for uptake of 100 ml O_2 during post-recumbency exercise than was the case before bedrest. This difference is statistically significant. The exercise factor is not altered by 9-α or bedrest as is shown in Table 26.

3. Tyramine Stimulation Test

The maximum rise in systolic arterial blood pressure resultant from central injection of 3 mgm tyramine is depicted in Table 27. The response was not altered by 9-α or bedrest. Similar

changes were noted in diastolic blood pressure. Baroreceptor slowing of heart rate was proportional to pressure rise. No alteration in pulmonary artery pressure was seen.

4. Valsalva Maneuver

Although this study was not performed on all subjects, the response shown in Figure 6 was typical of the subjects on whom it was performed. There was no qualitative difference in response after bedrest. However, quantitatively all subjects showed equal or greater "overshoots" on release of forced expiration after bedrest. Figure 7 shows the response of a patient with idiopathic orthostatic hypotension who was tested by methods identical to the study subjects. It is apparent that the response is quite different from the post-recumbency subject in that the "overshoot" is entirely absent.

C. Blood Volume Studies

Total blood volume results for the control and 9-α groups are tabulated in Tables 28 and 29. Mean and standard error for the entire group is presented for each day. In observing for alterations due to bedrest, Days 1 and 7 of ambulation and Day 1 of bedrest were used as control values. Days 2 and 6 of bedrest were considered as Week 1 and Days 11 and 15 as Week 2. The control group showed a fall in total blood volume during the first week of bedrest which was statistically significant. However, composite data from late in bedrest shows that the decrease was no longer significant. The changes in total blood volume in the

control group were almost completely accounted for by alterations in plasma volume which followed the same pattern (Table 30). The 9-α group showed no alteration in total blood volume or plasma volume (Table 31).

Table 32 and 33 depict the results from calculation of red blood cell volume. There was a decrease in red cell volume of doubtful significance during the initial week of bedrest. This was not apparent on late studies. No change was noted in the 9-α subjects.

Results of volume studies are summarized in Figure 8.

D. Balance Data

1. Water

Detailed data on water intake, output and balance for each control group subject is compiled in Table 34. Similar data is presented for the 9-α group in Table 35. Average daily water intake, output and balance for each subject in the control group is summarized by Table 36. Positive water balance was decreased by 300 ml during bedrest. This was statistically significant. The results obtained in the 9-α group were similar (Table 37). On comparison of the two groups there was no significant differences in intake, output, or balance during ambulation or during bedrest (Table 38).

2. Electrolytes

The predicted and analyzed sodium, potassium, calcium and magnesium contents of the formula diets are presented in

Table 39. In calculating balance data, analyzed content was used except for Subjects 1-7. Samples obtained on these subjects were contaminated, and it was necessary to use predicted contents.

Tables 40 and 41 tabulate detailed sodium balances for each subject in the control and 9-α groups, respectively. Cumulative balance for the ambulant and bedrest periods is presented in an uncorrected state as well as with a correction for 20 meq/day of extrarenal sodium loss.

The average 24-hour urinary sodium excretion for the two groups is summarized in Table 42. The two groups do not differ, and both show a highly significant increase in sodium excretion during bedrest. This is also depicted as average 24-hour sodium balance in Table 43. The mean day-to-day excretion of sodium and water for the control and 9-α groups are shown in Figure 9. From this graphic presentation, it is obvious that sodium and water loss are most profound during the initial days of bedrest. However, balance never returns to ambulant levels during the two weeks of bedrest. Figure 10 depicts the overall effect of bedrest on sodium and water balance.

Individual day-to-day potassium balance data for subjects in the control and 9-α groups are presented in Tables 44 and 45. The average 24-hour urine excretions and balances are summarized in Tables 46 and 47. There is a somewhat greater excretion of potassium and a tendency toward negative balance during bedrest. The changes in potassium balance were statistically significant in the 9-α group.

Detailed calcium balance data for subjects in the two groups is tabulated in Tables 48 and 49. The average 24-hour urine calcium excretion and balance is summarized by Tables 50 and 51. In both the control and 9-α groups, calcium excretion was increased and balance decreased during bedrest. These changes were of borderline significance in the control group and were insignificant in the 9-α group.

Magnesium balance data for the individual subjects in the control and 9-α groups is shown in Tables 52 and 53. Average 24-hour urinary excretion and average 24-hour balance of magnesium were unchanged by bedrest in either group as shown by the results in Tables 54 and 55.

Tables 56 and 57 contain the individual chloride balance data for the two groups. Since diets were constant and stool analyses not performed, only the average 24-hour chloride excretion is summarized. Table 58 shows a definite and statistically significant increase in urinary chloride during bedrest in both groups. This correlates well with the loss of sodium.

Individual phosphate balance data is compiled for the control and 9-α groups in Tables 59 and 60. As with chlorides, stool analyses were not made. Therefore, only average 24-hour phosphate excretion is summarized. Table 61 shows that there is an almost striking lack of variation in the mean excretion during the two phases.

Alterations in urine excretion of the various electrolytes by bedrest are summarized in Figure 11.

5. Urine Catecholamines

The values for urine norepinephrine (N.E.) and epinephrine (E.) are tabulated for each subject in the control and 9-α groups in Tables 62 and 63.

All norepinephrine results for each of the three weeks of study of each subject were averaged and are presented in Table 64. When the group means for each week are examined it can be seen that there is no significant alteration in catecholamine excretion as a result of bedrest. Neither is there a significant inter-group difference. Table 65 shows a similar summarization of urinary epinephrine results. These were also unaltered.

F. 17-Hydroxycorticosteroid Excretion

Table 66 and 67 tabulate all results of urinary 17-hydroxycorticosteroid analysis. No attempt has been made to evaluate them statistically. It is apparent by simple inspection that there is considerable individual variation within the normal range. Further, there is no consistent alteration associated with bedrest.

G. Aldosterone Excretion

Results for the control group are depicted in Table 68. There appears to be an inconsistent decrease in aldosterone excretion during bedrest. Similar data for the 9-α group is seen in Table 69. In this group a striking decrease in aldosterone

excretion is associated with the onset of drug therapy. In view of the similar sodium excretion seen in the control and treated groups, it would appear that the dose of 9-α used in these subjects depressed aldosterone secretion while substituting almost equivalently for its sodium retaining effect. Subject 11 was maintained on 9-α on resuming ambulation. It is interesting to note that his aldosterone excretion became twice as great as had been the case on the last bedrest day.

No definite conclusion can be drawn from the present aldosterone data. However, it is clear that aldosterone secretion is not depressed in any striking or consistent manner by bedrest.

H. Clearance Data

Tables 70 and 71 compile 24-hour urinary clearance of endogenous creatinine and phosphate as well as free water and osmotic clearance for Ambulant Day 1 and Bedrest Days 1 and 12 in control and 9-α treated subjects. In both groups there was a fall in endogenous creatinine clearance on the initial day of bedrest. This was not statistically significant. Phosphate clearance was unaltered. Perusal of free water and osmotic clearance reveals that water excretion during bedrest was mostly obligatory although there was a tendency for free water clearance to become less negative.

I. Urine Creatinine and Osmolality

Daily values for urine creatinine and osmolality are compiled for the control and 9-α groups in Tables 72 and 73.

The relative constancy of creatinine excretion attests to the reliability of urine collection procedures.

J. Blood Chemistries

Tables 74 and 75 tabulate results on various blood chemical analyses performed on Day 1 of ambulation and Days 1 and 12 of bedrest. The only noteworthy finding was the general depression in electrolytes in Subject 11 on Day 12 of bedrest. The reason for this finding is not clear. The subject did show a tendency to negative balance in all of the electrolytes shown to be low in serum. However, there was no subjective or clinical indication of the problem and results were not obtained until after release. Therefore, follow-up was not obtained.

Serum cholesterol and triglyceride results are tabulated in Table 76. There was no remarkable alteration due to bedrest. Although slight increases in cholesterol were noted in some subjects at the end of bedrest, the results were not striking. However, the diet was low in animal fat and high in unsaturated fatty acids. This would tend to prevent an increase in plasma lipids.

K. Hematologic Data

Table 77 and 78 compile the results of white blood counts, hemoglobin, hematocrit, and sedimentation rate done on Day 1 of ambulation and Days 1 and 12 of bedrest. The only significant alteration was a drop in hematocrit and hemoglobin on Day 1 of bedrest in some subjects. This blood was drawn in the early AM of the first day of bedrest and the most obvious explanation is

that each subject had undergone cardiac catheterization on the preceding day. The blood loss associated with this procedure was not excessive, but could easily account for some degree of change in hematocrit and hemoglobin.

VII. DISCUSSION

The reality of manned spaceflight has given great impetus to a total re-evaluation of the question of man's cardiovascular response to gravity and the absence of gravity. The basic questions relate to the validity of the classic concepts of cardiovascular adaptation and de-adaptation to gravity. The term cardiovascular deconditioning was coined by Keys and his group many years ago to describe the de-adaptation phenomena with its associated intolerance to the upright posture (107). The terms deconditioning and de-adaptation imply a deterioration of the cardiovascular system. Does this really occur? Is the problem due to failure of cardiovascular reflexes? Or is it due to depletion of intravascular volume? Is there any way of predicting at what point in orthostasis that vasodepressor syncope will occur? If these questions could be answered, then it would be possible to practice the most valuable type of space medicine, that is, preventive medicine.

The investigators who have been concerned with these questions have utilized two principal methods to simulate weightlessness. These are water immersion, which nullifies the hydrostatic column (189) and bedrest, which reduces its effect to approximately 1/7 G. It has also been found that the incidence of vasodepressor syncope is increased even by prolonged chair rest and by space cabin confinement for long periods, situations which have no effect on the hydrostatic column (25,114,116).

The basic effects of water immersion are said to be similar to those of bedrest. The diuresis associated with water immersion was described by Bazett in 1924 (18). This finding has been repeatedly confirmed by most investigators (19,71,74,75,76,100). Gowenlock also found that the inhibition of sodium excretion which occurs on standing in air after recumbency does not occur when the subject is made to stand in water after recumbency. That this change is hormonally mediated is suggested by the fact that aldosterone excretion is reduced when the subject stands in water (69). It is increased on standing in air. Water immersion has also been shown to produce plasma volume depletion and decreased tolerance to tilting (19,71,72,74,76,138,191,192,199,204).

The principal advantage of water immersion is said to be the fact that changes equivalent to about two weeks of bedrest can be induced in approximately six hours (72). This fact alone suggests that other mechanisms are at play besides simple nullification of hydrostatic pressure in the vascular system. One known problem inherent in most water immersion studies relates to the fact that a situation similar to negative pressure breathing exists (19). The vessels within the thorax are surrounded by air and are exposed only to air pressure. On the other hand, the vessels of the lower body are exposed to both ambient air pressure and water pressure. The net effect is that blood tends to be sucked into the chest. Since it is known that negative pressure breathing is associated with diuresis (14,26,57), it becomes problematical to decide whether

nullification of gravity effects or the negative pressure breathing effect is the predominant mechanism. Studies using total body immersion with mask pressure adjusted to water pressure levels have been performed. Under these circumstances diuresis has been less profound and changes in tilt tolerance equivocal (99). The use of positive pressure breathing during immersion has resulted in complete reversal of the cahnges noted by water immersion (101). In fact, tilt tolerance was said to be improved. These facts, plus the interference by water immersion with thermal exchange and sweating (192) suggest that this method is not an acceptable analogy of the physiology of weightlessness. Even though there is an extensive literature on this subject with many studies directed at preventing the deconditioning effects of water immersion, further review would seem to be of doubtful direct applicability to the question of the specific effects of reduced gravitational forces on the cardiovascular system.

Bedrest remains as the only earth based method which can be said to approach simulation of weightlessness. A number of bedrest studies of varying duration and type have appeared in recent years (25,41,42,102,145,146,187,198). Although performed many years ago, the most often quoted study is that of Deitrich, Whedon and Shorr (42). This excellent metabolic study was published in 1948 and has remained the classic study of the effects of bedrest. As relates to the cardiovascular system, these authors found that total blood volume decreased during the initial three weeks of

bedrest and then returned to a level only 5% below control values by 6-7 weeks of immobilized bedrest. They also found an increased tendency to vasodeprsssor syncope as early as one week after assuming bedrest. These authors concluded that "greater increases in extravascular fluid and greater progressive increases in venous engorgment" occurred during post-recumbency tilting. A marked decrease in exercise tolerance was also noted.

The more recent studies have been performed using periods of bedrest varying from 10 to 30 days. The results of these studies agree with Dietrich, Whedon and Schorr in that orthostatic intolerance was markedly increased after bedrest. Although none of these studies were metabolically controlled, they also confirm the occurrence of water and salt diuresis during bedrest. In contrast to the findings of Dietrich, Whedon and Shorr, plasma volume was found to decrease early in bedrest and to remain unchanged throughout bedrest in most studies (145,186,187,198,203,221). However, recent publications by Vogt, et al, suggest that technical problems may have nullified the significance of his earlier reports (195). His more recent and accurately performed studies show a return of plasma volume to normal at the end of 30 days (200). In those studies showing a decrease in plasma volume at the time of post-recumbency tilting there has been no correlation of tilt response with plasma volume decrease (145,187).

There has been considerable preoccupation with development of prophylactic measures before the mechanism of decreased orthostatic tolerance is clearly defined. For this reason, most studies have

been diluted by having small numbers of subjects in several groups. Each group trying a different form of prophylaxis. The lack of complete balance data on the subjects has precluded exacting analysis of results. However, much useful data has accumulated and methods have become more nearly standard. Much of the information accumulated to date has been covered in several recent review articles (33,44,73,115,136,137,148).

Although the metabolic control of the present study might be criticized from the viewpoint that hemodynamic studies were also performed, it was the aim of the authors to provide a well controlled hemodynamic study which would allow correlation of metabolic alterations with hemodynamic changes. The study also suffers the defect of other studies in that subjects were divided into control and 9-α treated groups. This division was clearly for the purpose of evaluating the protective effect of plasma volume maintenance. However, this maneuver was also quite valuable in defining the mechanism of decreased orthostatic tolerance after bedrest.

Perusal of results makes it quite apparent that 9-α had no effect in preventing vasodepressor syncope after bedrest. However, any beneficial effect might have been masked by the 50% incidence of vasodepressor syncope seen in each group during both pre- and post-recumbency tilt. It is also apparent that the cardiovascular effects of bedrest cannot be judged on the basis of vasodepressor syncope in this study as the incidence was unchanged after bedrest.

However, all subjects had tolerated a non-instrumented tilt before entry into the study and yet only half of them were able to tolerate the pre-bedrest tilt with arterial needle and cardiac catheter in place. This deleterious effect by vascular instrumentation may be reflex or psychic in origin. In any case, the resultant high incidence of vasodepressor syncope would undoubtedly mask an increase in frequency produced by bedrest alone. Similar effects of vascular instrumentation have been described by others (93,164,183).

One of the purposes of the present study was to determine if vasodepressor syncope could be predicted prior to its occurrence. From the data presented, it is apparent that the usual parameters of arterial blood pressure and heart rate were not of great value in this prediction. Pulse pressure did tend to show greater narrowing in subjects who subsequently developed syncope. Alterations in pulmonary artery pressure provided no assistance in this matter. On the few occasions where right atrial pressure was measured, it appeared that vasodepressor syncope was preceded by a decrease in pressure which was not seen in the stable subject. It also appeared that, as a group, the more stable subjects (those who tolerated 18 minutes of tilt) showed a smaller decrease in cardiac output, central blood volume and stroke volume, as well as a greater rise in heart rate than was true in the less tolerant subjects. On an individual basis these group differences were less apparent.

Of the observations made, the activity of the vasomotor waves appeared to have a significant bearing on the occurrence of vasodepressor syncope.

Phasic variations in blood pressure and heart rate were first observed in hypotensive acidotic dogs (133). These so-called Mayer waves were subsequently proven to be under the control of the arterial chemoreceptors since they could be abolished by correcting the acidosis (7). With the elucidation of the baroreceptor function of the carotid sinus, another type of blood pressure wave was recognized which was free of chemoreceptor control (132). This vasomotor wave is generated by variations in afferent impulses from the arterial baroreceptor to the vasomotor center in the medulla. In the stable vascular system such as is seen at supine rest in the present study, these afferent impulses show little variation and minimal vasomotor activity is observed in the systemic blood pressure. However, assumption of the tilt or upright posture, with the associated redistribution of blood and diminished stretch of the baroreceptors, results in an alteration of the number of afferent impulses being sent to the vasomotor center. This center integrates the impulses and initiates processes designed to stabilize blood pressure. Sympathetic tone is increased while parasympathetic tone is decreased. The net effect results in vasoconstriction and a rise in heart rate. Heart rate responses are known to be more rapid than pressure changes which may account for the lag in the pressure waves

behind the heart rate wave (24). As pressure rises, the process is reversed. This occurs repeatedly at a rate of 2-8 times per minute. In order for this system to be functional, both components of the autonomic system must be operative (66). Thus, when pressure rises, the decrease in heart rate is mediated by the parasympathetic nervous system, but when pressure falls, the increase in heart rate is mediated primarily by the sympathetic nervous system. As redistribution of blood continues during tilting, tachycardia increases and the heart rate waves become damped. The pressure waves continue to be visible at this time. This is due to the fact that the carotid sinus mechanism is less effective in opposing heart rate increase than it is in altering peripheral vascular resistance (24). However, it has been noted that the vasomotor pressure waves also disappear under circumstances of maximal vasoconstriction. This is seen particularly well in Raynaud's disease (132). In the present study the pressure waveforms showed a definite decrease in amplitude and frequency during the two minutes prior to the occurrence of syncope. In addition to suggesting a potential means of predicting the occurrence of syncope, these findings would seem to point to the presence of maximal vasoconstriction immediately prior to syncope.

Although the occurrence of vasodepressor syncope during 70° tilt could not be used as a measure of the effect of bedrest, the hemodynamic parameters which were evaluated in the present study were strongly influenced. Cardiac output, central blood volume

and stroke volume showed significantly greater decreases after bedrest. At the same time, heart rate and peripheral vascular resistance were significantly elevated. When the latter findings are coupled with the unaltered post-recumbency response to tyramine injection and Valsalva maneuver, it is apparent that the sympathetic nervous system was intact and functioning normally. Further support for this fact is the knowledge that catecholamine excretion was not affected by bedrest. Thus, diminished sympathetic nervous activity is not responsible for the failure to maintain pre-recumbency levels of cardiac output, central blood volume and stroke volume during the post-recumbency tilt.

Prior studies have suggested that the decreased orthostatic tolerance seen after periods of bedrest may be due to plasma volume depletion (186). Depletion in plasma volume by bedrest is said to be due to increased stimulation of the low pressure central baroreceptors (volume receptors). Impulses from these receptors result in inhibition of A.D.H. and aldosterone (5,59,110,149). Thus, water and sodium diuresis would occur at the expense of plasma volume. There is not complete agreement on the existence of this system (40). In the present study, the decrease in aldosterone excretion induced by bedrest was inconsistent. A.D.H. was not measured. However, there was a significant sodium and water diuresis in both the control and 9-α treated groups. In the control group this was associated with an initial fall in plasma volume. No such decrease occurred in the 9-α treated group.

At the time of the post-recumbency tilt, plasma volume was statistically unchanged from pre-recumbency levels in both groups. Thus, it would appear that the diminished orthostatic tolerance seen in the study subjects cannot be attributed to a decrease in vascular filling induced by plasma volume depletion during bedrest.

Another possible explanation would be the occurrence of greater venous pooling during post-recumbency tilting. Although this cannot be totally dismissed, it has been shown that venous tone is increased minimally and transiently by the stimulus of gravity (32,166,190). Thus, there was little protective effect even before bedrest. Since the hydrostatic column is no greater after bedrest than before, it would be unlikely that its effect would be more pronounced after bedrest.

A more plausible explanation for the profound hemodynamic effects of the post-recumbency tilt may be postulated on the basis of the data obtained. In both the treated and untreated group there was a marked increase in sodium and water loss during recumbency. The cause of this diuresis is probably related to the increase in central blood volume associated with recumbency with resultant stretch of the low pressure baroreceptors. The role of third factor in this diuresis is speculative (29,175). Based on an average decrease in water balance of 250 ml/day during bedrest, it may be assumed that net water deficit during the entire period of bedrest was in the range of 2-3 liters. Since plasma volume was unaltered at the end of bedrest, it must be assumed that the

fluid was lost from the extravascular compartments. The sequence of events would include loss of plasma water by diuresis with eventual replacement of this loss by the iso-osmotic equivalent from the extravascular compartments. Thus, extravascular hydration would be sacrificed in order to maintain optimal vascular filling. Contraction of the extravascular compartment would eventually stimulate extravascular volume or osmolar receptors with a resultant slowing or inhibition of diuresis and saluresis. A new level of counterbalanced activities of the low pressure intrathoracic and extravascular receptors would thus be achieved. However, extravascular dehydration would persist. This would be accompanied by a lowered tissue pressure which, in turn, would decrease resistance to capillary filtration. During the post-recumbency tilt, the hydrostatic pressure would cause a greater flow of fluid out of the vascular system than had been the case before bedrest. This would occur because of the resultant increase in capillary filtration pressure. Guyton has shown that a decrease in tissue pressure of 1 mm. Hg. increases the filtration of fluid out of capillaries 1.2 times as much as a 1 mm. Hg. increase in venous pressure (82). Thus, at the actual time of performing the post-recumbency tilt study, there was not only the same degree of venous pooling in the lower extremities that had been present during the pre-recumbency tilt, but also a large and progressive transudation of plasma water into the tissues of the lower extremities. This transudation would be greater than normal as a result of virtual dehydration of the extravascular space. Progressively diminishing vascular

filling pressure would then result in a falling venous return to the right heart. Central blood volume would continue to decrease as would stroke volume. Normal baroreceptor mechanisms would increase heart rate in an only partially successful attempt to maintain cardiac output. At the same time increased sympathetic tone due to baroreceptor activation would initially maintain blood pressure as nearly optimal as possible. However, this very increase in peripheral resistance would tend to further impede blood flow and thereby reduce venous return to a greater extent. The potential deleterious effects of marked vasoconstriction have been well described (122). At some critical point the lower limit of central vascular filling would be reached signaling the imminent occurrence of failure of blood flow. It is postulated that the low pressure baroreceptors would sense this impending catastrophe and in a last ditch effort to maintain flow would inhibit the adrenergic sympathetic system and stimulate the sympathetic cholinergic system to reduce peripheral vascular resistance (15,16,196). The sudden increase in capacity of the poorly filled vascular system would result in a dramatic drop in blood pressure. This would diminish cerebral blood flow and result in syncope. At the same time, the baroreceptors would have accomplished their purpose of maintaining blood flow. Thus, as noted in other studies, stroke volume and cardiac output would not change remarkably during syncope from the levels existent immediately before syncope (65,212,218).

This hypothesis is supported by the greater increase in limb volume noted during tilting after bedrest (84). In our laboratory we have found that when lower extermity volume is measured by impedance plysthmography during tilting it does not return to baseline for 30-60 minutes after resuming the supine posture. If all of the increase in limb volume was due to venous pooling, return to baseline should be rapid. In addition, it was noted in the present study that there was a failure to augment stroke volume during post-recumbency supine exercise. As previously noted, the resting values were obtained prior to tilt testing and exercise performed approximately 20 minutes after tilt-down. Thus, the most logical explanation for failure to augment stroke volume above pre-tilt resting values lies in the supposition that a portion of the pre-tilt plasma volume was no longer available. Vascular filling was then sub-optimal during exercise. Therefore, it is likely that the resting stroke volume at the time of the exercise was actually lower than that used for comparison with exercise values. Thus, normal augmentation may have been masked. Reduction in stroke volume and cardiac output is known to occur after a reduction in total blood volume (34). This effect may persist for extended periods of time (38,193). The behavior of the vasomotor waves also tends to confirm the hypothexized mechanism, in that they suggested the presence of maximal vaso-constriction immediately prior to syncope.

The possibility of an increased degree of plasma water extravasation during post-recumbency orthostasis has been suggested by a number of investigators (42,115,198,202). It should be noted that Vogt's conclusions were quite similar to those reached in the present study (198). A recent report by Vogt confirms the decrease in extracellular and total body water during bedrest (202). The methodology for this type of study is fairly complex, with many pitfalls. Thus, more extensive evaluation is needed.

The present study does not exclude the possibility of loss of myocardial mass and strength during bedrest. It has been shown that negative nitrogen balance occurs during bedrest as a result of muscle catabolism (42). This may well contribute to fluid extravasation by decreasing lower extremity muscle tone. However, the heart is a perpetually active muscle and it seems doubtful that myocardial catabolism occurs during prolonged exposure to the hypogravic state. The failure to augment stroke volume and the resultant greater rise in heart rate seen during post-recumbency 50 watt exercise in the present study can be explained by the above hypothesis. However, it could be equally well explained by diminished myocardial contractility with a higher left ventricular end-systolic volume. This requires further evaluation.

The effects of the small doses of 9-α given to subjects in the present study were manifest by: marked suppression of aldosterone excretion; plasma volume maintenance without expansion; and a less profound saluresis during the initial few days of bedrest.

However, the effects of bedrest on sodium and water balance were not altered by 9-α when the entire bedrest period was considered. In addition, diuresis was primarily obligatory even in the 9-α group on the first day of bedrest. There are several possible explanations for this observation. The dose of 9-α used may have been so small as to merely serve to counterbalance the drug and bedrest induced decrease in aldosterone production. The possibility also exists that the saluresis seen as a result of bedrest is due to causes other than aldosterone inhibition. The work of Stahl, previously commented on, provides a possible non-hormonal mechanism (176). How this may relate to the third factor is presently problematic (29). As noted, 9-α reduced early bedrest saluresis without altering net saluresis during the total bedrest period. Thus, it would appear that the effect of bedrest on the volume receptors, renal arteriolar pressure, or third factor finally became predominant over the sodium-retention effects of 9-α. The fact that average sodium and water balance was the same in both groups while no plasma volume decrement was seen in the 9-α group is of interest. It allows one to speculate that the degree of extravascular dehydration may have been more severe in the treated group during the early phase of bedrest. However, had the subjects been tilted at that time, it is doubtful if the overall response of the two groups would have differed since the difference in extravascular hydration would have been compensated for by the difference in vascular filling. Irrespective of these

early differences, after two weeks of bedrest the two groups did show equalization in terms of plasma volume, sodium and water excretion and hemodynamic response to stress. Thus, it is obvious that small, continuously administered, doses of 9-α are of no value in maintenance of cardiovascular homeostasis during bedrest. This dosage would not be expected to behave in any other manner during prolonged weightlessness. Since the institution of the present study, a report has appeared in which 2.0 mgm of 9-α-fluorohydrocortisone was given on each of the last two days of a six day bedrest period. Four subjects were studied. As might be expected with this dose, positive water balance and sodium retention resulted in an increase in plasma volume (182). No orthostatic responses were reported. If 9-α were to be used by astronauts, its use late in spaceflight would be the most logical method of re-expanding the plasma volume. Another study has shown a remarkable increase in Na^{22} space shortly after intravenous 9-α administration as well as after ten days of treatment with 1.0 mgm/day given orally (170). However, there was evidence of increasing sodium diuresis after three days. These authors also noted the occurrence of negative potassium balance which was aggravated by supplementary sodium. Schirger also found early expansion of intravascular and extracellular fluid volume which returned toward basal levels with long term treatment (167). Thus, continuous administration of 9-α during spaceflight, whether at high or low dose levels, would be of doubtful value. As in the case of

Seager's study (170), we noted a tendency toward the development of hypokalemia in a number of our 9-α treated subjects. This is coupled with our finding of significantly negative potassium balance in the 9-α group. Hypokalemia might well aggravate the problem of orthostatic intolerance (55,159). Thus, any consideration of use of this compound by astronauts must be judged with caution. Much more extensive evaluation should precede such usage.

Other methods of prophylaxis against the development of post-hypogravic orthostatism have been studied extensively in recent years (41,117,139,146,147,184,201,205,206). Of the methods evaluated, two show great promise. The use of lower body negative pressure has been shown to simulate the effects of the orthostatic posture on the vascular system (181). The application of lower body negative pressure to recumbent subjects appears to prevent the alterations in body fluids that occur during bedrest (117, 139,184). As a result, better orthostatic and exercise tolerance was found in the L.B.N.P. subjects than in controls. The use of leotards during post-recumbency tilting also prevents the undesirable effects of the orthostatic posture (205).

In addition to the alterations in post-recumbency exercise response which have been noted, ventilatory equivalent was significantly increased after bedrest. Thus, the energy cost of providing metabolic requirements for oxygen was greater after bedrest. The mechanism involved is not clear. Lung perfusion (cardiac output) was significantly less during post-recumbency

exercise. However, oxygen consumption was not altered in comparison to pre-recumbency studies. If the change in ventilatory equivalent were flow related, a fall in oxygen consumption might have been expected rather than a rise in ventilation. Thus, the possibility of an alteration in oxygen diffusion capacity cannot be excluded. An alternative explanation might be an increase in dead space ventilation.

As noted in prior studies (42,102,198), calcium balance tended to become negative during bedrest in our subjects. It is interesting to speculate on the relationship of the increased urinary excretion of calcium and the passage of a calcium oxylate stone by one of our subjects. This problem is well known in patients who are immobilized for lengthy periods (109). However, the presence of another disease process in this subject tends to cloud the issue in spite of the negative past history of genito-urinary disease. The question of development of renal stones in healthy subjects at bedrest must be answered by future studies which are directed solely at the question of calcium metabolism. It should be noted that some investigators doubt that renal calculi will represent a problem during spaceflight (153). The failure to show bedrest induced alterations in urinary 17-hydroxycorticosteroids in the present study agrees with the findings of others (106,198).

Since it has been shown that lack of exercise increases the risk of coronary heart disease (177), the possibility of alterations in serum cholesterol and triglyceride was evaluated in the

current study. No significant alteration was noted during the brief period of inactivity encompassed by this study. This question will require eventual evaluation as many astronauts are in the fourth decade of life.

Other than the alteration in serum potassium in one 9-α subject, no significant change in blood chemistry occurred. Neither was there any alteration in the hematologic picture that could not be explained on the basis of blood withdrawals. Derived red cell mass showed no change. However, no evaluation of post-recumbency hemolysis was attempted in the current program.

SUMMARY

The effects of two weeks of absolute horizontal bedrest were evaluated in twenty subjects, ten of whom received 0.1 mgm of 9-α-fluorohydrocortisone twice daily during bedrest. Metabolic and hemodynamic parameters were studied. The following conclusions are based on the results of the present study as well as a review of the literature.

1. Orthostatic intolerance is increased by the hypogravic state. Under certain circumstances of gravitational stress this could represent a major hazard to astronauts.
2. The increase in orthostatic intolerance is probably due to the development of extravascular dehydration during exposure to the hypogravic state. The possibility of decreased myocardial contractility cannot be excluded and requires evaluation.

3. The occurrence of vasodepressor syncope may possibly be predicted prior to its occurrence by external biosensors developed to detect arterial vasomotor waves. These waves tend to disappear prior to syncope. Further study of the relationship of these waveforms to syncope would appear to be indicated.
4. 9-α-fluorohydrocortisone would be of doubtful value if given chronically during spaceflight. It may provide some orthostatic protection if given during the terminal 48 hours of spaceflight. However, the problems associated with drug-induced negative potassium balance must be further evaluated.
5. The risk of renal calculus formation during exposure to the hypogravic state needs careful evaluation.
6. It is doubtful that methods will be available for prevention of extravascular dehydration by the time of the first manned moon-flight. However, the astronaut may be given much protection by the use of an anti-gravity suit during any interval when G-stress approaches that of earth. Such a situation is expected to occur on moon lift-off of the L.E.M., as well as on return to earth.

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